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NATIONAL INSTITUTE OF SCIENCES OF INDIA

NOTICE

The National Institute of Sciences of India, since its inception in 1935, has been publishing regularly the *Proceedings* every year of which twenty volumes were published till January, 1955. It contained miscellaneous articles on Physical and Biological Sciences, as well as important official proceedings of the Institute.

The Publication Advisory Board of the Institute had been considering for some time past whether it would be possible to have the *Proceedings* published in two Parts, that is, one Part on *Physical Sciences* and the other Part on *Biological Sciences* and to have the official proceedings published in the Year Book, on the lines of the publications of the Royal Society. An effort was made last year in this direction and the articles on Physical Sciences and Biological Sciences were published in alternate issues of the *Proceedings* (Vol. XX, 1954). On a report from the Publication Advisory Board that with the articles coming in for publication by the Institute it will be possible to have the *Proceedings* published in two parts, the Council decided that with effect from Volume XXI (1955) the *Proceedings* should be published in two Parts, that is, Part 'A' for Physical Sciences and Part 'B' for Biological Sciences and they be issued monthly in alternate months.

In view of this new form of publication, the titles of the two Parts of the *Proceedings* which will be issued from 1955 will be:

Proceedings, National Institute of Sciences of India, Part A, Physical Sciences, and

Proceedings, National Institute of Sciences of India, Part B, Biological Sciences.

The Year Book will be an annual publication which will contain the official proceedings of the Institute as were hitherto published in the *Proceedings*.

As regards the division of the *Proceedings* into Parts 'A' and 'B', the Volume and Year numbers will be the same for the two Parts, with their own Title Page, page numbering and Index, both the Parts starting with No. 1, as Physical Science, Part A, No. 1 and Biological Science, Part B, No. 1 and aiming to have yearly six issues for each Part.

The following abbreviations are suggested for use in References:

For *Proceedings*, National Institute of Sciences of India, Part 'A', Physical Sciences—*PNISIPS*.

For *Proceedings*, National Institute of Sciences of India, Part 'B', Biological Sciences—*PNISIBS*.

J. M. SEN,
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Calcutta,
26th January, 1955.

PHOTOPERIODISM IN RICE

IV. EFFECTS OF SHORT DAY LENGTH ON THREE MEDIUM-EARLY VARIETIES OF RICE OF UTTAR PRADESH

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(Communicated by P. Parija, F.N.I.)

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INTRODUCTION

The photoperiodic responses of different varieties of paddy have been markedly different as reported by workers in this field. Pan (1936) working with certain Chinese varieties found that different short-day photoperiods bring about 10 per cent increase in the average height of the treated plants over the controls. Fewer culms were formed in the treated plants of early and medium-maturing varieties. He also observed that the period of maturity in the treated plants was cut short, shortening being more conspicuous in the late-maturing varieties than in the early ones.

Fuke (1931) working on certain Japanese varieties of paddy showed that short days were most effective on freely tillering plants, having 7-9 leaves. Treatments given to more advanced plants, a month prior to heading time, were much less effective. Such treatments were equally ineffective either when provided to very young plants, which had not even entered the 7-9 leaves stage, or to older ones, which were but 16 days behind the natural heading time.

In U.S.A., Beachell (1943) studied the effects of different short-day treatments, administered at different stages of plant development, in 10 varieties of rice grown under field conditions. He concluded that the varieties, based on their reactions to these treatments, could be divided into two groups. (1) 'Sensitive', and (2) 'Less Sensitive'.

The effects of short and long photoperiods on early varieties of U.P. (Misra, 1955a), on late varieties of Bihar (Saran, 1950) and Bengal (Sircar, 1942 and 1946) and on one spring variety of Orissa (Misra, 1954c) reveal the fact that different varieties of Indian paddy respond differently to different photoperiods. The present investigation was, thus, aimed at finding out the photoperiodic responses of 3 medium-early varieties of paddy of the State of Uttar Pradesh.

MATERIAL AND METHODS

The three different varieties of paddy used for experimentation in this work were as follows:

- (1) T. 3, a selection from Basmati of Dehra Dun district,
- (2) T. 12, a selection from Hansraj of Unao district, and
- (3) T. 21, a selection from Chawal of Rampur State.

Seeds were first sown in small nursery pots (15"×5") and segregated into half-a-dozen lots. A week after germination, the seedlings belonging to different lots were subjected to 10-hour photoperiodic exposures for 3, 4, 5 and 6 weeks respectively. One of the lots which received the above treatment for 6 weeks in the seedling stage was further provided with this short-day treatment until panicle emergence.

The photoperiodic treatments consisted of a daily 10-hour exposure to natural daylight in the field from 8.0 a.m. to 6.0 p.m. For the remaining 24-hour cycle, the potted plants were removed to a well ventilated dark room. The seedlings were subsequently transplanted into other pots (11"×10") and allowed to grow in the open till maturity. Controls were maintained side by side. Except for differences in the photoperiodic exposures, all plant lots were grown under identical conditions of factor intensity. A brief report was made in this direction in an earlier publication (Misra, 1953).

EXPERIMENTAL RESULTS

Heading in Plants.

Data gathered on the time of panicle emergence are presented in Table I. These observations were subjected to statistical analysis as reported in Table II. At the time of panicle emergence in the control plants, representatives from the control and treated series were photographed to exhibit differences in their growth behaviour (Figs. 1 and 2).

TABLE I

Time from sowing to ear emergence of the main shoot.

Varieties	No. of days from sowing to ear emergence (Average of 24 plants)			
	T.3	T.12	T.21	Mean
<i>Treatments</i>				
Controls	106.25	99.30	108.70	104.75
S. Day for 3 weeks	106.30	107.55	111.15	108.33 (3.58)
S. Day for 4 weeks	107.70	108.90	112.05	109.55 (4.80)
S. Day for 5 weeks	109.75	110.25	113.75	111.25 (6.50)
S. Day for 6 weeks	112.45	111.85	115.85	113.38 (8.63)
S. Day prolonged till ear emergence ..	128.00	121.25	121.55	123.60 (18.85)

S.E. of a treatment mean = 0.45; C.D. at 5% of a treatment mean = 1.26.

S.E. of an individual mean = 0.78; C.D. at 5% for comparing two individual means = 2.19.

Sowing date: June 18, 1949; transplanting date: June 25, 1949. + indicates earliness; -- indicates delaying effect. Figures in brackets indicate difference from controls.

TABLE II

Analysis of variance.

Source of variation	D.F.	S.S.	M.S.	F.	5% F.	1% F.
Varieties (V)	2	239.22	119.61	39.73**	3.13	4.92
Treatments (T)	5	3,132.47	626.49	208.13**	2.35	3.29
Interaction (V×T)	10	351.90	35.19	11.69**	1.97	2.59
Error	72	222.32	3.01			
Total	89	3,945.91				

** Indicates significance at 1% level.

A perusal of the data on ear emergence (Tables I and II) clearly shows that there is a gradual delay in the heading of plants as the duration of short-day treatment increases in the seed-bed and thereafter. With 3 weeks of short-day treatment, delay in the ear emergence averaged 0.5, 8.25 and 2.45 days respectively for the three different varieties of paddy, viz., T.3, T.12 and T.21. With 6 weeks of similar treatment, the heading time of these three varieties was delayed by 6.20, 12.55 and 7.15 days respectively. When the short-day treatment was prolonged till ear emergence, the delay in the commencement of heading was much more (Table I).

Although short-day treatments greatly delayed the ear emergence in all the three varieties of paddy, no inhibitory effect by way of complete suppression of some of the ears of the main shoot was observed in the prolonged short-day series. There was another important finding. Of the three varieties of paddy experimented upon, T.12 responded most to the photoperiodic treatments and variety T.3 the least. It may here be noted that T.12 is comparatively an early variety.

Some Morphological Characters.

Observations on the number of tillers, number of leaves and the height of plants, belonging to different treated and control series, were made at four regular intervals in their life-cycle. The final records are presented in Table III. It may be noted that the trend of all such observations was almost the same at the different stages of plant growth.

TABLE III

Certain morphological observations following short-day exposure of seedlings for different periods of three paddy varieties

Paddy varieties	Controls	Short-day exposures for				
		3 weeks	4 weeks	5 weeks	6 weeks	Prolonged till heading
		No. of tillers per plant				
T.3 ..	4.55	4.20	4.45	4.65	4.90	1.70
T.12 ..	4.30	3.00	3.15	3.25	3.05	1.40
T.21 ..	4.05	3.05	3.65	3.60	3.85	2.15
Mean ..	4.30	3.41	3.75	3.83	3.93	1.75
		No. of leaves per plant				
T.3 ..	15.90	16.35	16.55	16.90	17.45	6.85
T.12 ..	14.10	9.10	10.20	11.00	10.35	8.05
T.21 ..	14.15	11.20	11.25	12.65	13.70	9.10
Mean ..	14.71	12.21	12.67	13.51	13.83	8.00
		Height per plant in cm.				
T.3 ..	124.90	123.15	126.50	128.20	132.65	83.85
T.12 ..	135.40	157.70	150.95	157.35	152.25	104.35
T.21 ..	137.25	146.95	143.90	146.30	151.45	107.50
Mean ..	132.51	142.60	140.45	143.95	145.45	98.56

A careful examination of the data (Table III) would reveal the following points of interest:

(a) None of the short-day treatments seemingly induce any significant difference in tiller number in plants of paddy variety T.3 as compared with untreated controls. In the other two varieties of plants, a decrease in this respect was well evident, and more so in variety T.12. In all the three varieties, the prolonged short-day treatment, however, reduced the number of tillers, rather considerably.

(b) Number of leaves slightly increased following all the seed-bed treatments on plants of paddy variety T.3. In varieties T.12 and T.21 (specially in T.12), leaf number, contrariwise, decreased. There was a marked decrease in this respect in plants of all the three varieties, when subjected to prolonged photoperiodic treatment. These observations were more or less similar to those obtained for tillers.

(c) All photoperiodic treatments ranging from 3 to 6 weeks initiated an increase in the height of plants belonging to varieties T.12 and T.21. In variety T.3, however, the treatments for 5-6 weeks only effected such a change. In the series of plants, of all the three varieties, subjected to prolonged photoperiodic treatment, the height invariably decreased.

Grain Yield.

The grain yield was recorded in the usual way by weighing the sundried fully mature grains. Average weight of grains per plant is presented graphically in figure 3.

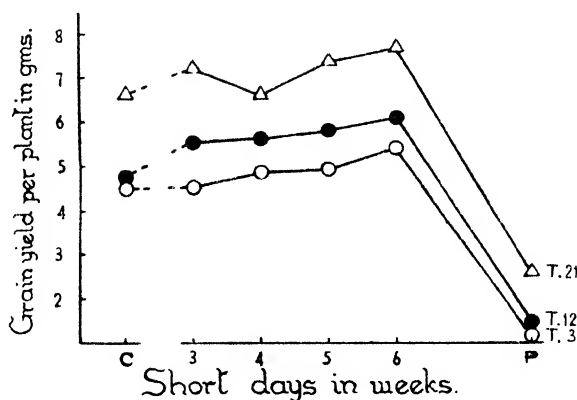
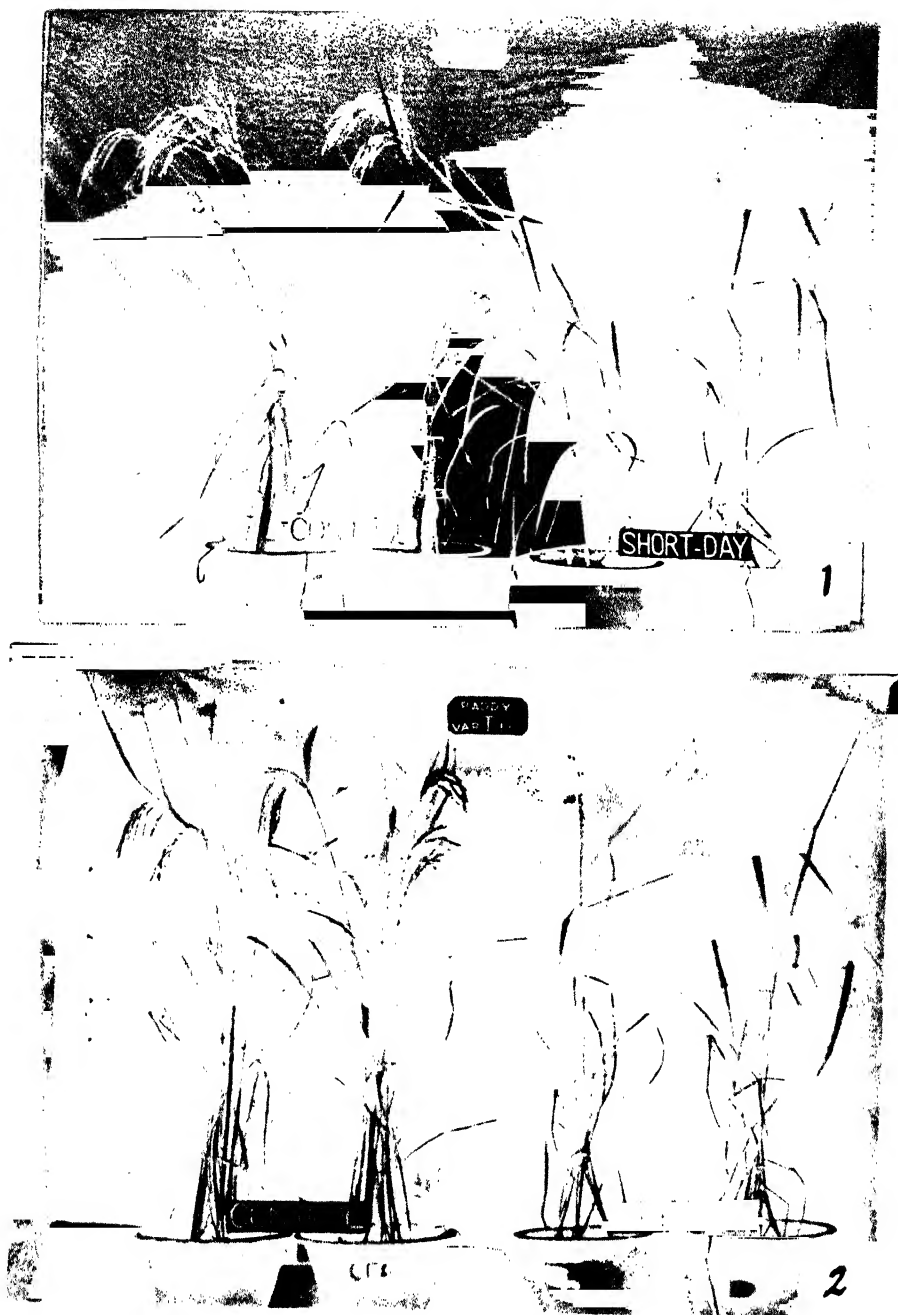


FIG. 3. Grain yield per plant in gms. of the three medium-early varieties T.3, T.12 and T.21 when the 7-day old seedlings were subjected to 10 hour short days for 3, 4, 5, 6 weeks and till ear emergence. The grain yield in prolonged treatment set (P) is considerably less in comparison with the control set (C) receiving full length of natural day.

The data on grain yield were subjected to statistical analysis. It is found that short-day treatment of 3, 4, 5 and 6 weeks durations did not induce any significant difference in yield over the controls in variety T.3. In variety T.12, 5 to 6 weeks treatments initiated higher yields. Similar increases in yield are obtained in variety T.21, following 6-weeks photoperiodic treatments.

The overall effect of treatments in the seed-bed, with regard to increases in grain yield, was significant for all the three different varieties, being 14.3 per cent



FIGS. 1, 2. Earliness and copious flowering in the control plants exhibiting luxuriant growth in the medium-early varieties of rice, T.3 and T.12 as against the prolonged short day (10 hr.) treated plants which are still continuing a poor vegetative growth. Seed sown June 18. Photographed October 15.

over the controls for the lot receiving short-day treatment for 5 weeks and 21.1 per cent for the one similarly treated for 6 weeks. A highly significant decrease in yield to an extent of 67.3 per cent compared with the controls was, on the other hand, obtained for the experimental lot treated in the like manner till the time of ear emergence.

Components of Yield.

The components of yield, such as, the number and length of panicles, the number of spikelets and grains per panicle, the percentage of grains set per panicle, and the absolute weight (wt. of 1,000 grains) of grains are presented in Table IV. The results of statistical analyses of these data are shown in Table V.

The following observations are made on an analysis of the data presented in Tables IV and V:

(a) Of the three varieties of paddy experimented upon, two behaved slightly differently than the third. The number of panicles produced under the influence of 3, 4, 5 and 6 weeks short-day treatments was not significantly different in varieties T.3 and T.21 than those borne by the controls. In variety T.12, however, these very treatments brought about a significant decrease in this respect. When, on the other hand, the short-day treatment prolonged till ear emergence, a conspicuous decrease in the panicle number was obvious in all the three varieties of plants.

(b) There was no marked difference in the length of the panicle belonging to the control plants and the ones that received the short-day treatment for 3, 4, 5 and 6 weeks respectively in the varieties T.3 and T.21. An increase in this direction was, however, observed under similar treatments in variety T.12. The short-day treatment prolonged till ear emergence, contrariwise, significantly decreased the length of the panicle, in all the three varieties.

(c) The average number of spikelets per panicle greatly increased in the lots receiving short-day treatments for 3, 4, 5 and 6 weeks respectively. Of these, the 6-weeks short-day treatment was more effective for all the three different varieties, T.3 exhibiting the least response and T.12 the maximum. Short-day treatment prolonged till ear emergence, reduced the number of spikelets considerably in all the three varieties of paddy.

(d) The number of grains per panicle significantly increased in plants of varieties T.12 and T.21 by short-day treatments of 3, 4, 5 and 6 weeks duration. In variety T.3, however, no significant difference from the controls was observed in this respect in any of the treated lots. When the short-day treatment continued beyond the 6th week stage until ear emergence, an adverse effect on grain formation followed. The effect was very marked and highly significant.

(e) The effect of the short-day treatments on the three varieties of paddy was an increase in the percentage of grain setting, particularly in the lots receiving the treatment for 5 and 6 weeks. It is of interest to note that although in the lot where the short-day treatment continued till heading time, the spikelets and grains per panicle considerably decreased relative to controls, the percentage grain setting per panicle was not adversely affected. It was rather high for all the three varieties of paddy.

(f) The absolute weight of a thousand grains did not appreciably alter under the influence of any of the short-day treatments for any of the three varieties of paddy used for experimentation.

TABLE IV

Effect of short days on the characters of panicle, spikelets and grains

Paddy varieties	Controls	Short-day exposures for				
		3 weeks	4 weeks	5 weeks	6 weeks	Prolonged till heading
<i>No. of panicles per plant</i>						
T.3 ..	4.45	3.95	4.25	4.35	4.00	1.65
T.12 ..	3.70	2.75	2.65	2.80	2.60	1.30
T.21 ..	3.30	2.80	2.90	2.80	2.85	1.85
Mean ..	3.81	3.16	3.26	3.31	3.15	1.60
<i>Length of panicle in cm.</i>						
T.3 ..	23.20	22.54	22.58	23.30	24.24	14.20
T.12 ..	20.18	22.98	22.56	22.74	23.88	12.64
T.21 ..	21.62	23.90	21.42	21.46	21.86	15.02
Mean ..	21.66	23.14	22.18	22.50	23.32	13.95
<i>No. of spikelets per panicle</i>						
T.3 ..	70.36	80.54	72.08	74.80	85.10	42.38
T.12 ..	89.70	131.26	131.68	120.80	132.64	67.88
T.21 ..	99.38	113.76	110.20	112.88	116.78	66.18
Mean ..	86.48	108.52	104.65	102.82	111.50	58.81
<i>No. of grains per panicle</i>						
T.3 ..	50.76	59.68	58.66	57.94	64.84	32.14
T.12 ..	63.60	93.54	98.28	96.42	108.14	54.44
T.21 ..	78.92	98.60	90.60	99.30	103.08	57.14
Mean ..	64.44	83.94	82.51	84.55	92.02	47.90
<i>Percentage of grains set per panicle</i>						
T.3 ..	72.14	74.09	81.38	77.45	79.95	75.83
T.12 ..	70.90	71.26	74.63	79.81	81.52	80.20
T.21 ..	79.41	86.67	82.21	87.96	88.26	86.34
Mean ..	74.15	77.34	79.40	81.74	83.24	80.79
<i>Weight of one thousand grains in gms.</i>						
T.3 ..	20.12	19.46	19.58	19.60	21.00	21.26
T.12 ..	20.98	21.60	21.74	21.94	22.00	21.16
T.21 ..	25.68	26.34	25.30	26.56	26.38	24.58
Mean ..	22.26	22.46	22.20	22.70	23.12	22.33

TABLE V
Analyses of variance of the data of components of yield

Source of Variation	D.F.	S.S.	M.S.	F.	5% F.	1% F.
<i>No. of panicles per plant</i>						
Varieties ..	2	23.68	11.84	40.82**	3.13	4.92
Treatments ..	5	42.48	8.49	29.27**	2.35	3.29
Interaction (V × T) ..	10	6.11	0.61	2.10*	1.97	2.59
Error ..	72	21.05	0.29			
Total ..	89	93.32				
<i>Length of panicle</i>						
Varieties ..	2	13.54	6.77	4.31*	3.13	4.92
Treatments ..	5	954.77	190.95	121.62**	2.35	3.29
Interaction (V × T) ..	10	58.48	5.84	3.71**	1.97	2.59
Error ..	72	113.10	1.57			
Total ..	89	1,139.89				
<i>No. of Spikelets per panicle</i>						
Varieties ..	2	28,460.41	14,230.20	85.16**	3.13	4.92
Treatments ..	5	29,856.93	5,971.38	35.73**	2.35	3.29
Interaction (V × T) ..	10	3,410.47	341.04	2.04*	1.97	2.59
Error ..	72	12,029.97	167.08			
Total ..	89	73,757.78				
<i>No. of grains per panicle</i>						
Varieties ..	2	21,641.47	10,820.73	91.62**	3.13	4.92
Treatments ..	5	20,368.76	4,073.75	34.49**	2.35	3.29
Interaction (V × T) ..	10	2,058.62	205.86	1.74	1.97	2.59
Error ..	72	8,503.32	118.10			
Total ..	89	52,572.17				
<i>Weight of one thousand grains</i>						
Varieties ..	2	516.81	258.40	195.75**	3.13	4.92
Treatments ..	5	9.05	1.81	1.37	2.35	3.29
Interaction (V × T) ..	10	25.36	2.53	1.91	1.97	2.59
Error ..	72	95.24	1.32			
Total ..	89	646.46				

* Significant at 5% level.

** Significant at 1% level.

DISCUSSION

From the foregoing results, it is clear that short-day treatments of 10 hours per day beginning with 1-week-old seedlings for varying durations of 3, 4, 5 and 6 weeks respectively bring about a gradual delay in the first panicle emergence in each of the three varieties of paddy. When the treatment is continued till heading, the delay in panicle emergence is observed to be much more. The photoperiodic responses of these medium-early varieties of paddy fall in line with the early varieties of U.P., where also a delay in the ear emergence was observed under such treatments (Misra, 1955*a*). Working with early paddy varieties of Bengal, Sircar and Ghosh (1947) noted a similar delay in the onset of heading. This relationship of short photoperiodic treatments with the lengthening in the vegetative period of paddy plants does not appear to be of universal occurrence. Sircar and Parija (1949) did not observe such a relationship with two other varieties of early paddy. Treating two early and eight late varieties of paddy with long and short photoperiods for a fortnight, Kar (1946) concluded that high temperature associated with short day lengths would induce earliness and low temperature or long day lengths would, contrariwise, produce retardation effect. The observations made in the present investigation give evidence but contrary to Kar's generalisations. In the three medium-early varieties of U.P. paddy grown under the prevailing warm climate, short photoperiods instead of inducing earliness bring about a marked delay in heading.

The response of these medium-early varieties to prolonged photoperiodic treatments is rather different than of the late varieties. Using the same photoperiods as in the present investigation until the usual heading time, Misra (1954*a* and *b*) observed a marked earliness in the ear emergence of four early-winter and four late-winter varieties of U.P. paddy.

It is clear that exposure of plants of three medium-early varieties of paddy to short photoperiods for 3 to 6 weeks delayed the ear emergence on an average by 3 to 9 days. Short-day treatments are, therefore, of no avail in obtaining an early crop of paddy. There is, however, an increase in grain yield to an extent of 8 to 21 per cent on an exposure of plants to the same short-day treatments. This increase in yield evidently owes to the larger number of grains formed per panicle and to better setting.

It is found that the number of panicles formed per plant as a result of short-day treatments is actually less than the controls and the absolute weight of grains is seemingly unaffected. All the same if there is no danger of flood or other misfits of nature, the crop can yield about 21 per cent more on standing for a period of about 10 days more than the normal. This increase in yield will certainly prove of importance to paddy growers.

While studying the effects of 24-hour long light periods on these medium-early varieties of paddy, a delaying effect on ear emergence was noticed (Misra, 1955*b*). On the basis of their flowering behaviour in relation to length of day, these medium-early varieties of paddy may, thus, aptly be classified as 'intermediates'. They flower within a definite range of the length of the day and blossom but less readily when photoperiodic exposures are either too short or too long (Allard, 1938 and Allard and Garner, 1940).

SUMMARY

The effect of 10-hour photoperiods was studied on the flowering behaviour of three medium-early varieties of U.P. paddy. The experiments were conducted as pot culture. Altogether 5 different types of short-day treatments were given to different lots of paddy plants in the seed-bed. These included 3, 4, 5 and 6 weeks of short-day treatments and in the fifth lot such a treatment continued till ear emergence.

There was a gradual delay in ear emergence on the main shoots of plants, as the duration of short-day treatment increased in the seed-bed and thereafter.

The short-day treatments for different durations of 3 to 6 weeks respectively, retarded the formation of tillers, leaves and panicles, but, contrariwise, enhanced plant elongation and panicle growth. The number of spikelets and grains per panicle also increased. Although the absolute weight of grains remained unaffected, there was a general improvement in grain setting due to these short-day treatments. The grain yield was consequently higher than the controls.

Short-day treatments prolonged till the usual heading time, caused an all-sided adverse effect, excepting on the absolute weight of seeds.

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THE INVOLUTION OF THE THYMUS OF THE LIZARD, *CALOTES VERSICOLOR* (DAUD.)

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INTRODUCTION

Although the thymus resembles lymph nodes in its physiological and pathological reactions (Gyllensten, 1953), it differs from them in its morphology, histology and embryonic development. One of the striking points of resemblance of thymus and lymph nodes is in their involution with age (Andrew and Andrew, 1948). The prevailing view held by many investigators (Hammar, 1927; James, 1939; Smith and Louise, 1941; Gregoire, 1943; Baillif, 1949; Feldman, 1951; Smith *et al.*, 1952*b*) is that during involution of the thymus there is shrinkage accompanied by replacement of parenchymal tissue by connective and adipose tissues. Andrew and Andrew (1948) have reported that the atrophic process of the cervical lymph nodes of rat involves loss of parenchymal tissues, without any replacement by connective or adipose tissues, leading to the formation of large cavities of 'astonishing size'. According to Baillif (1951) the destruction of lymph nodes involves dissolution of lymphocytes, stromal cells and fibres, thus forming dilated cystic nodes which are at first multilocular, but later reduced to an unilocular sac. The present study was undertaken to determine if the thymus of *Calotes versicolor* behaves as the lymph nodes do during involution. It was found that in several respects the thymus of this species resembles on the one hand the thymus of mammals, while on the other it recalls the condition seen in amphibia.

MATERIAL AND METHODS

No attempts were made to rear the lizards in the laboratory but the animals were prepared for study directly after collection from the field. Examination was made of the contents of the alimentary canal in every case to make sure that the lizards had been well fed and all those that showed a paucity of food were discarded. For the purpose of this study twenty-four lizards weighing from 6 grams to 77 grams were used. The thymus was dissected out and fixed in Zenker's formol-acetic and

Bouin's fluids. Sagittal sections in paraffin were cut at $7\ \mu$ and $10\ \mu$, and stained in Delafield's haematoxylin-eosin, Mallory's triple and Heidenhain's iron-haematoxylin. Shorr's differential stain as modified by Svihla (see Hartman, 1944) was also used.

OBSERVATIONS

To begin with it will be appropriate to review the morphology of the thymus of *Calotes versicolor* (specimens weighing 22–56 grams) with special attention to the structures concerned in involution. The size, shape and histological appearance of the thymus vary greatly depending on the age and weight of the animals. The thymus of *Calotes versicolor* is made up of an anterior oval and a posterior more rounded lobe separated by interlobar connective tissue (Fig. 1). Each lobe is surrounded by a fibrous capsule. There is an outer cortex and inner medulla. The cortex is composed of medium and small sized thymocytes (lymphocytes). The medulla consists of thymocytes, reticular cells forming medullary cords, basophilic granulocytes (foamy cells) unicellular Hassall's corpuscles, multinucleated plasmodial masses and sinusoids. All the intra-thymic blood vessels are surrounded by perivascular connective tissue which is continuous with the capsule.

Group 1. (Specimens weighing 6 to 18 grams.)

The thymus of seven specimens in this weight group was examined. In these young animals the thymus is small. The cortex can be differentiated from the medulla, but medulla appears more extensive (Fig. 1). The capsule is uniformly thick and is made of fibroblasts and a few mast cells. Thymocytes predominate in the cortex and are closely packed. The medulla consists of many unicellular Hassall's corpuscles with vesicular nuclei indicating a relationship to the reticular cells. Their cytoplasm shows concentric striations (Fig. 10). The multinucleated plasmodial masses arise by fusion of reticular cells. Generally these masses undergo degeneration. Dissolution of cytoplasm takes place first at the periphery and it later extends inwards. Lacunae are thus formed with central degenerating tissues (Figs. 11 and 12). The lacunae are small and lined by flattened reticulum resembling the endothelial wall of a blood vessel. Basophilic granulocytes are few. No macrophages are seen.

Group 2. (Specimens weighing 22 to 56 grams.)

Twelve specimens were examined. The thymus is large and has reached its maximum size. The capsule is compact, but thin, except at the region where the trabeculae enter the parenchyma of the gland. The mast cells are numerous and large, having coarse basophilic granules. The cortex is compact, more extensive than medulla, and is composed chiefly of thymocytes (Fig. 2). The thymocytes are more numerous than in the younger animals (group 1). Their accumulation is particularly great in the cranial and caudal ends of the gland. Mitotic figures are abundant in the cortical region, but degenerating thymocytes are also common. Degeneration consists in the breaking down of thymocytes into deeply staining spheroidal bodies (Fig. 13) resembling in all respects the 'Tingible Körper' of lymphocytes (Andrew and Andrew, 1948). These finally undergo karyolysis and are disposed off by phagocytes. Thus there appear many clear rounded patches in the cortex where groups of thymocytes have undergone complete degeneration (Fig. 2). Pycnotic thymocytes also occur all over the cortex. There are a few abnormal mitoses in anaphase showing chromosomal bridges (Fig. 13).

The medulla is loose and contains medium and small thymocytes, unicellular Hassall's corpuscles, multinucleated plasmodial masses, lacunae with or without degenerating tissues, granulocytes and macrophages (Figs. 12, 15, 16). The trabe-

culae, though narrow, extend deep into the parenchyma. The reticular cells have vesicular nuclei and are predominant in this region. The multinucleated plasmodial masses are undergoing cytolysis forming large lacunae (Figs. 12 and 16). The unicellular Hassall's corpuscles are generally scattered throughout the gland, but are found in conspicuously large numbers in the medulla. Their cytoplasm is acidophilic and exhibits concentric striations (Figs. 14 and 16). A few corpuscles have vacuoles in their cytoplasm (Fig. 14).

The basophilic granulocytes are generally in groups and occur near the multinucleated plasmodial masses and blood vessels. Each cell is large and swollen with many basophilic granules (Fig. 15). In preparations stained with iron-haematoxylin these cells appear yellow, but after Mallory's triple stain they are coloured pale blue and appear light green when stained with Shorr's method. Some granulocytes have few granules but others have many, depending perhaps on the physiological condition of the cell as well as of the gland. The granulocytes are large in number and also the granules appear numerous.

The macrophages are found in large numbers in the vicinity of the lacunae containing degenerating masses. They are polymorphic cells with vesicular nuclei indicating that they are probably derived from reticular cells. Their cytoplasm contains black, brown or golden yellow pigment and phagocytosed inclusions (Fig. 16).

Group 3. (Specimens weighing 58 to 77 grams.)

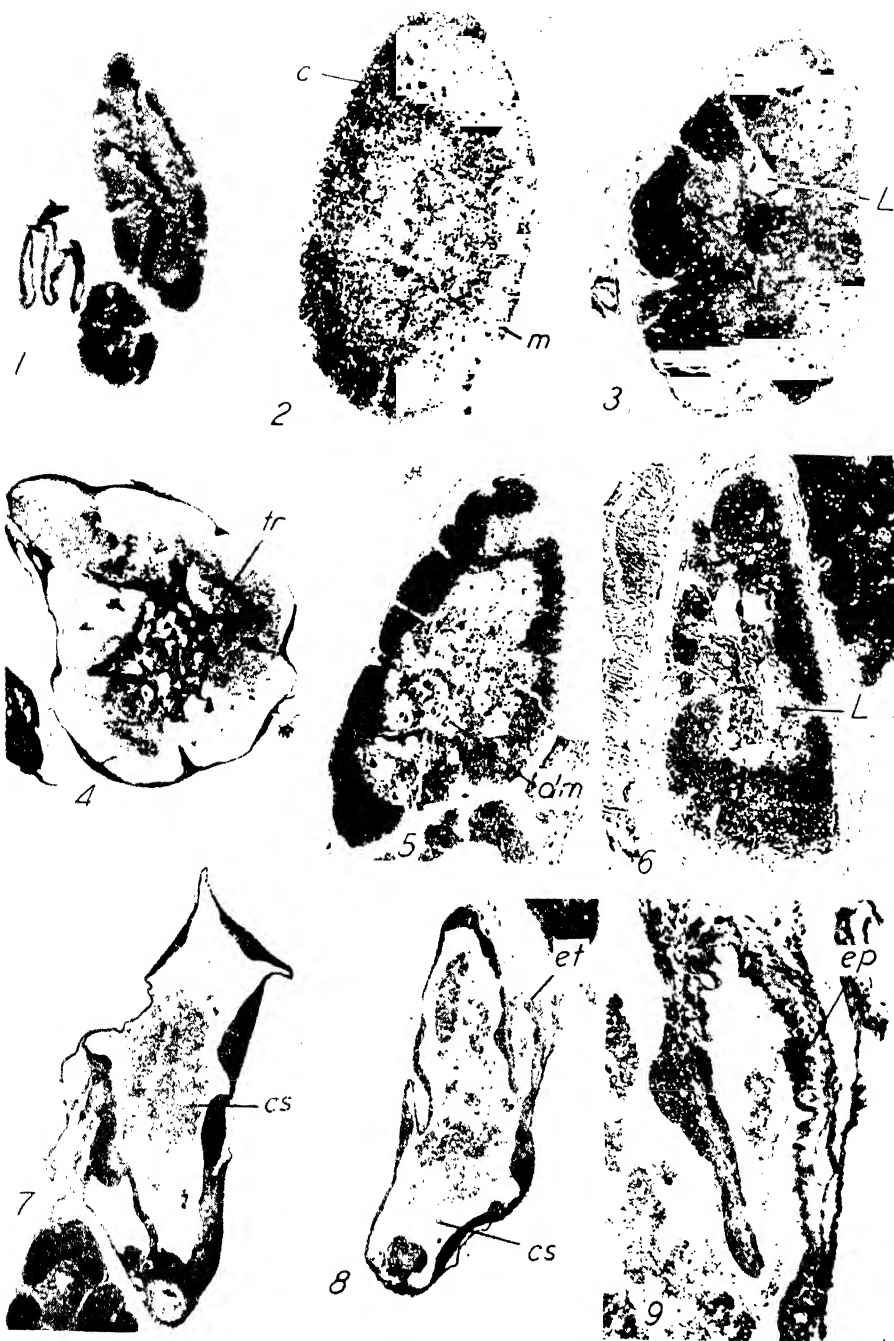
In the three specimens examined in this weight group, the conspicuous features of the thymus are the increase of connective tissue in the parenchyma and the presence of large spheroidal and ovoidal cavities in the medulla with or without degenerating masses (Figs. 3, 5 and 6).

The capsule is thick as compared with that in the preceding groups. This thickening is due to increase in number of fibres and their separation. The mast cells are large in number and size. Many trabeculae enter into the parenchyma from all sides of the gland and form a network in the medullary region (Figs. 3, 4, 5 and 6).

The medulla is extensive and the medullary cords reach the capsule at several places (Figs. 3 and 6). Thus there is a tendency to hyperplasia of reticular cells as a result of which the cortex is greatly reduced (Figs. 3 and 5). The lacunae have increased in number and size (Figs. 5) and are lined not only by flattened reticulum but also by the fibres (Figs. 6 and 17). Many of them now open into lymph vessels and thus are aptly called sinusoids, though they have originally arisen as products of liquefaction of the plasmodial masses. These plasmodial masses are partially or completely absorbed as involution proceeds (Figs. 5 and 6). As there are many thymocytes in blood vessels of the capsule and as the trabeculae, which are highly vascularized, enclose the cortical tissues, it seems reasonable to conclude that the thymocytes have migrated into the blood stream and that their place is taken by hypertrophied medulla.

Group 4. (Specimens weighing 54 and 56 grams.)

Among the animals studied there were two which weighed 54 and 56 grams, but which displayed in their thymus such outstanding characteristics that they have been dealt with separately. The thymus in these animals has undergone complete involution. The most striking feature is the presence of a large cavity occupying practically the entire gland except for a few patches of cortical tissue and reticular cells (Figs. 7 and 18). The cystic space is lined by flattened reticulum. In certain places the cavity extends up to the capsule (Fig. 7). It seems to involve not only the medulla but also large areas of the cortex and is filled with fluid coagulum



containing free thymocytes, a few reticular cells, unicellular Hassall's corpuscles and macrophages (Figs. 7 and 18). The basophilic granulocytes are few in number.

It is interesting to note the presence of an epithelial tubular structure opening into the cystic space of the thymus (Fig. 8). It is lined externally by the capsule and internally by vacuolated cuboidal epithelial cells (Fig. 9). This epithelial lining is continuous with the internal lining of the cavity of the thymus (Figs. 8 and 9). In the lumen of the tube are thymocytes which have migrated from the cystic space of the thymus.

DISCUSSION

The involution of the thymus of *Calotes versicolor* offers many points of interest, particularly in its resemblance to lymph nodes. It has been known for a long time that during sexual maturity and old age the lymph nodes undergo atrophy. During this process the parenchymal elements of the nodes are replaced by connective and adipose tissues (Hellman, 1930; Krumbhaar, 1942). According to Andrew and Andrew (1948) and Baillif (1951) on the other hand, the atrophy is due, not to a replacement of the original cell elements by new ones, but to a total loss of cells resulting in cavity formation. The thymus of *Calotes versicolor* involutes by a process essentially similar to this.

Andrew and Andrew (1948) have stated that it is possible to identify with considerable accuracy the nodes of young, middle aged and senile rats. An application of their observations to the findings in *Calotes versicolor* seems possible. For in this animal, the changes in the thymus so characteristically reflect their age groups that I have ventured to suggest a classification into (a) juvenile, (b) middle aged (c) old and (d) senile forms, depending on the structural changes in the thymus. Thus animals weighing 6 to 18 grams are considered as juvenile, 22 to 56 grams middle aged, 56 to 77 grams, old and 54 to 56 grams as senile animals. In *Calotes versicolor* during the early period of involution there is a certain amount of infiltration of connective tissue from the capsule into the parenchyma of the thymus (Figs. 3, 5 and 6); but later there is a definite loss of both cortical as well as medullary tissues leading to the formation of large cavities (Figs. 5, 6 and 7) in the thymus of old and senile animals. The loss of parenchymal tissue cannot be attributed either to infiltration of or to replacement by connective and adipose tissues as stated by Hammar (1927), Baillif (1949), Feldman (1951) and Smith *et al.* (1952), because no such replacement or infiltration of tissues is seen during involution. On the contrary, the loss of parenchymal tissues is so great that it leads to the formation of large cavities where there is neither overgrowth nor replacement of other tissues. Figures 7 and 8 which represent stages of almost complete involution, are interesting, in that they do not show any evidences of overgrowth or infiltration. The involution of the thymus of *Calotes versicolor*, therefore, strongly recalls the atrophic process of lymph nodes of rats described by Andrew and Andrew (1948) and Baillif (1951).

In the normal thymus of rat, the capsule is thin (Baillif, 1949) but at the time of involution it becomes a thick coat of loosely matted sheath of fibres. In the lymph nodes of this animal also, a similar change has been reported (Andrew and Andrew, 1948). It is a matter of interest that in *Calotes versicolor*, the capsule of the thymus which remains thin in the normal thymus, becomes a thickened sheath of fibres during involution (Fig. 6).

The flat cells lining the wall of the sinuses of the lymph nodes have been described by Ribbert (1907) as endothelium. According to his view the sinuses have been derived from the lymphatics of the nodes. Downey (1915 and 1922) sees intimate anatomical relations between the flat cells of the sinus wall and reticular cells of the lymph nodes and postulates that 'these sinuses develop not from pre-formed embryonic lymphatics but arise as independent blind cavities in the mesenchyme of the lymph node primordium'. The cavities later fuse with afferent

and efferent lymph vessels. Jordan and Looper (1928) described in the thymus of the box turtle a closely graded series of the formation of the sinuses from the degeneration of reticular cells. Andrew and Andrew (1948) are of the opinion that the cystic spaces in the lymph nodes of rats also arise 'as areas of cell and fiber loss in the more compact tissues of medullary cords or of the cortex'. The present author has observed that in the thymus of *Microhyla ornata* (in press) the sinusoid spaces (lacunae) arise by dissolution of the hypertrophied reticular cells.

The origin of the sinusoid spaces in the thymus of *Calotes versicolor* conforms to the process described by Downey (1915 and 1922) in the mammalian lymph nodes, by Jordan and Looper (1928) in the thymus of the box turtle and by Andrew and Andrew (1948) in the cervical lymph nodes of rats. These spaces arise by liquefaction of the hypertrophied reticular cells. At first the cells form multinucleated plasmodial masses (Fig. 11). Frequently dissolution of their cytoplasm takes place at the periphery and extends inwards forming lacunae lined by flattened reticulum with central degenerating masses (Figs. 5, 6, 11 and 12). These lacunae in older forms open into lymph vessels and are called sinuses (Fig. 17). They widen up gradually in old and senile animals forming large cystic spaces (Figs. 6 and 7).

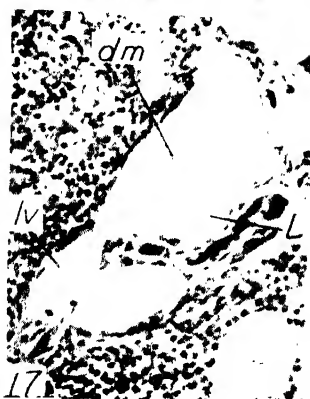
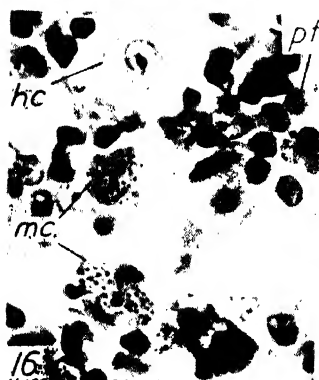
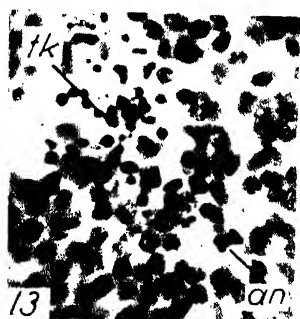
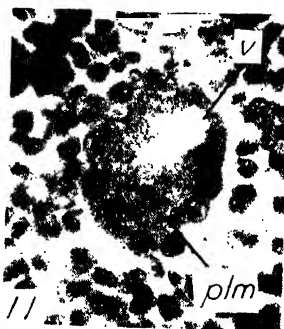
The unicellular Hassall's corpuscles have been described as sarcoytes by Mayer (1888), Schafer (1893) and Pensa (1902). Hammar (1905), Jordan and Looper (1928) and Fabrizio and Charipper (1941) consider them as modified reticular cells. In the thymus of *Calotes versicolor* these corpuscles resemble to a great extent the reticular cells. Jordan and Looper (1928) describe in the thymus of the box turtle the occurrence of vacuoles in the sub-peripheral region of the corpuscles; later, with the coalescence of the vacuoles, an exoplasmic area is separated from the endoplasmic nucleated portion. I find in my material vacuoles in the peripheral region of the corpuscle but have not noticed their coalescence as in that of the box turtle thymus (Fig. 14).

The presence of basophilic granulocytes during involution is of great interest. Jordan and Looper (1928) believe that the appearance of the granulocytes in the thymus of the box turtle is stimulated by tissue destruction and regard them as modified lymphocytes. Baillif (1949) in thymus and Andrew and Andrew (1948) in cervical lymph nodes of the rat consider these granulocytes as modified mast cells. A detailed account of the granulocytes of the thymus of the rat is given recently by Loewenthal and Smith (1952) who call them lipid laden foamy cells.

The basophilic granulocytes are found in large numbers in middle aged and old forms of *Calotes versicolor*. As they occur near the multinucleated plasmodial degenerating masses their appearance is perhaps due to stimulation offered by the degenerating tissues as suggested by Jordan and Looper (1928). Their morphology suggests a resemblance with the foamy cells described by Loewenthal and Smith (1952). The number of granules depends upon the physiological condition of the cells. As these cells are situated far from trabeculae and as they differ from mast cells in their shape it is probable they are derived from thymocytes and not from mast cells as stated by Andrew and Andrew (1948) and Baillif (1949).

Maximow and Bloom (1932) believe that the macrophages are derived from reticular cells. Baillif's (1949) contention is that the intralobular macrophages of the thymus alone are derived from the epithelial stromal cells. Smith *et al.* (1952a) are of the opinion that macrophages, reticular cells and lymphocytes are the precursors of the adipose cells and probably they enter into some transient histiocytic condition before becoming fully differentiated fat cells.

Macrophages are present in large numbers in the thymus of middle aged *Calotes versicolor* in the vicinity of the sinusoid spaces (Fig. 16). They are large, polymorphic cells with vesicular nuclei containing cytoplasmic granules (brown, black or yellow) and vacuoles. As they are found in the medullary region of the thymus and as their nuclei resemble those of reticular cells it can be said that they are derived from reticular cells as suggested by Maximow and Bloom (1932) and Baillif (1949).



Whether they are the precursors of adipose cells as stated by Smith *et al.* (1952a) is difficult to say.

The presence of epithelial inclusions in the lymph nodes has been noted by Ries (1897), Hellman (1930), Comes (1938) and Andrew and Andrew (1948). These inclusions are normally found in the lymph nodes of the young forms, but tend to disappear with increasing age. In *Calotes versicolor* I have found an epithelial tubular structure in the involuting thymus (Figs. 8 and 9). I am unable to develop any definite view in regard to its origin due to the lack of more complete information.

There is a strong suggestion that the thymus of *Calotes versicolor* behaves like the cervical lymph nodes of rats (Andrew and Andrew, 1948) during involution.

SUMMARY

1. In the thymus of juvenile forms there is a preponderance of medulla over cortex; in middle aged animals, cortex is more extensive; in old age there is again hyperplasia of medulla with a corresponding decrease in cortex.
2. A conspicuous feature in the involuted thymus is the presence of large cavities formed by the dissolution and atrophy of thymo-reticular cells without any replacement by connective or adipose tissues.
3. The capsule thickens during involution. This thickening is effected by increase in number as well as by separation of the capsular fibres.
4. The sinusoid spaces arise by liquefaction of the multinucleated plasmoidal masses and they communicate with lymph vessels.
5. The unicellular Hassall's corpuscles are derived from reticular cells of the thymus. Some show the presence of vacuoles in their cytoplasm prior to their liquefaction.
6. The basophilic granulocytes (foamy cells) are formed from thymocytes. They are present in large numbers in middle aged and old forms. They occur near the degenerating plasmoidal masses. The number of granules in these cells depends upon the physiological condition of the cell as well as of the gland.
7. The macrophages are found during involution. They are the transformed reticular cells.
8. Occasionally an epithelial tubular structure is present in the involuted thymus.
9. It is suggested that the thymus of *Calotes versicolor* behaves like the cervical lymph nodes of rats during involution.

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EXPLANATION OF FIGURES

Plate II

- FIG. 1. -Sagittal section of the thymus of juvenile form (weight, 12 grams) showing an anterior oval and a posterior round lobe. Note the preponderance of medulla over cortex. Heidenhain's haematoxylin. $\times 28$.
- FIG. 2. -Sagittal section of the anterior lobe of the thymus of middle aged form (weight, 34 grams) showing accumulation of thymocytes in the cranial and caudal ends of the gland. H.H. $\times 49$.
- FIG. 3. -Sagittal section of the anterior lobe of the thymus of old form (weight, 58 grams). The hyperplasia of medullary region and the presence of lacunae are seen. H.H. $\times 49$.
- FIG. 4. -Sagittal section of the anterior lobe of the thymus of old form (weight, 62 grams), showing the network of trabeculae in the medullary region. Shorr's differential stain. $\times 49$.
- FIG. 5. -Sagittal section of the anterior lobe of the thymus of old form (weight, 71 grams), showing the presence of lacunae with degenerating masses. H.H. $\times 49$.
- FIG. 6. -Sagittal section of the anterior lobe of the thymus of old form (weight, 77 grams), showing the presence of cavities in the medullary region. H.H. $\times 49$.
- FIG. 7. -Sagittal section of the anterior lobe of the thymus of senile form (weight, 54 grams), wherein the gland has undergone complete involution. Note presence of large cavity occupying the entire gland. H.H. $\times 49$.
- FIG. 8. -Sagittal section of the anterior lobe of the thymus of senile form (weight, 54 grams), showing the presence of an epithelial tube. H.H. $\times 49$.
- FIG. 9. -A portion of the thymus of senile form (weight, 54 grams) enlarged to show the lining of the epithelial tube. The epithelium is cuboidal and is continuous with the internal lining of the cystic wall. The thymocytes in the epithelial tube have migrated from the cystic space of the thymus. H.H. $\times 130$.

Plate III

- FIG. 10.—Unicellular Hassall's corpuscles showing concentric striations. Their nuclei are vesicular. (Thymus of juvenile form; weight, 12 grams.) H.H. $\times 1400$.
- FIG. 11.—A portion of the medulla of the thymus of juvenile form (weight 12 grams) enlarged to show the plasmodial mass with the dissolution of its cytoplasm at the periphery. H.H. $\times 1120$.
- FIG. 12.—A portion of the medulla of the thymus of middle aged form (weight, 34 grams) enlarged to show the formation of lacunae in the plasmodial masses. Mallory's triple. $\times 480$.
- FIG. 13.—A portion of the cortex of the thymus of middle aged form (weight, 34 grams) to show the presence of 'Tingible Körper' and pyknotic thymocytes. An aberrant anaphase is also seen. H.H. $\times 1120$.
- FIG. 14.—Unicellular Hassall's corpuscle showing a vacuole in the cytoplasm. Mallory's triple. $\times 1800$.
- FIG. 15.—A portion of the medulla of the thymus of middle aged form (weight, 34 grams) enlarged. A basophilic granulocyte with granules is seen near a lacuna. Note the basophilic granules also. Mallory's triple. $\times 1150$.
- FIG. 16.—A portion of the medulla of the thymus of middle aged form enlarged (weight, 34 grams). Three macrophages with their pigment granules are seen around a lacuna. A unicellular Hassall's corpuscle is also seen. H.H. $\times 1150$.
- FIG. 17.—A portion of the medulla of the thymus of old form (weight, 58 grams) enlarged to show the opening of the lacuna into a lymphatic vessel. H.H. $\times 310$.
- FIG. 18.—A portion of the thymus of the senile form (weight, 54 grams) enlarged. The reticular cells, thymocytes and unicellular Hassall's corpuscles are torn away from the wall of the thymus. H.H. $\times 360$.

KEY TO LETTERING

<i>an</i>	Aberrant anaphase.
<i>bgc</i>	Basophilic granulocytes.
<i>c</i>	Cortex.
<i>cs</i>	= Cystic space.
<i>dm</i>	= Degenerating mass.
<i>ep</i>	= Epithelium (Columnar).
<i>et</i>	Epithelial tube.
<i>hc</i>	= Hassall's corpuscle.
<i>L</i>	Lacuna.
<i>lv</i>	Lymphatic vessels.
<i>m</i>	Medulla.
<i>mc</i>	= Macrophages.
<i>plm</i>	Plasmodial mass.
<i>pt</i>	= Pyknotic thymocytes.
<i>tk</i>	= Tingible Körper.
<i>tr</i>	Trabecular network.
<i>v</i>	Vacuole.

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STUDIES IN THE VITAL CAPACITY OF NOKTE NAGAS OF TIRAP FRONTIER DIVISION (NORTH-EASTERN FRONTIER)

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INTRODUCTION

Hutchinson (1848, quoted by Myers, 1925) concluded after extensive investigations that the variations in the measurements of vital capacity of healthy individuals could be only due to the variations in the physical builds of the persons. Dreyer (1919) pointed out that the variation in the vital capacity might be due to racial factor. He also devised formulae to correlate vital capacity with standing height, sitting height, weight, chest circumference, etc. He argued that the sitting height was a better index of vital capacity than the other measurements. West (1920) found best correlation between vital capacity and body surface area as calculated from Du Bois's formula and suggested that the ratios of $\frac{\text{vital capacity}}{\text{standing height}}$ and $\frac{\text{vital capacity}}{\text{surface area}}$ should be used as normal standards. Attempts were also made to correlate vital capacity with trunk measurements (Jackson and Lees, 1929). Wilson and Edwards (1921) supported the theory of racial factor after getting strikingly low results in the coloured children. Similarly, Foster and Hsieh (1923) obtained much lower values both in the Chinese males and females as compared with the American males and females.

Whatever the causative factor or factors of variations may be it is always true that the vital capacity of lungs is a test for determining the physical fitness and efficiency since this is measured by a maximum expiratory effort after a maximum inspiration by the lungs. Its variation in different subjects from the standards of one race who are living under the identical climatic and other conditions is taken to be due to poor physique or unhealthy with diseases, particularly those of the respiratory system.

Undoubtedly, therefore, the vital capacity test is of great physiological importance and it is also a valuable guide in the medical diagnosis of diseases of heart and lungs. In Great World War I, this test was used in the U.S.A. for assessing the physical fitness and efficiency of individuals for the purpose of recruitment to various military services. It was also one of the tests used for recruitment to the Royal Air Force.

For determining the vital capacity standards for normal individuals of different countries, correlations had been worked out between vital capacity on one hand and different physical measurements on the other. Statistical methods were also used by some workers. Hutchinson (*loc. cit.*) initiated the work in England and thereafter the problem was extensively worked out in England and U.S.A. Foster and Hsieh (*loc. cit.*) and McCloy (1927) worked on the Chinese, Satake and Sato (1938, quoted by Telang and Bhagwat, 1941) determined the vital capacity of the Japanese and Myers (*loc. cit.*) worked on the Philipinos. A brief description of the studies carried out in India is given below for comparative study.

VITAL CAPACITY OF INDIANS

To determine the Indian standards vital capacity tests were mainly made on the medical students of Bombay, Madras and Bengal. Bhatia (1929) observed a racial difference in Bombay after getting much lower results there. Krishnan and Varad (1932) working on 103 male South Indian students observed the average of 2.86 litres or only 67 per cent of the average British and American standards. The authors put forward the arguments that the lower standards obtained were not due to racial differences but due to influence of hot climate resulting in low metabolism and less tendency to work. The same authors (1933) repeating the estimations on 260 medical students obtained the average vital capacity of 3.05 litres or 75 per cent of the American standards. Mason (1932) found that the average vital capacity in 587 South Indian women subjects was 2.15 litres which was 76 per cent of the average standard of American women. She suggested series of anthropological measurements and study of the weight of the lungs to find out how much of the differences was due to racial factor. Chatterjee (1933) worked out the average vital capacity of Bengali College students at the age of 19 years along with other physical measurements and he found the normal value of 2.72 litres, statistical data and correlation ratios with other physical measurements were not worked out. Calcutta University (1934) reported that the average vital capacity of 522 Bengali College students between the ages of 17 to 20 years was 3.0 litres, the coefficient of variation of vital capacity was between 16-22% and the variability of the direct ratio between vital capacity and body surface area was minimum. There is a big difference in these two results of Bengali College students, investigations for both of which were carried out under the auspices of Calcutta University and at about the same time. De and De (1939) observed the average vital capacity of Bengalees was only 2.721 litres which was lower than the results obtained in Bombay and Madras but same with the finding of Chatterjee (*loc. cit.*). Telang and Bhagwat (1941) with the main object of investigating the correlation between the vital capacity and other physical measurements by statistical methods estimated the vital capacity of 172 Bombay medical students. The average was 2.95 litres. Standing height and sitting height were found to be least variables, weight was more so than the surface area. Vital capacity was found to be least correlated with age and polidisi but significantly correlated with other measurements to a greater or lesser degree. Reddy (1933) recorded observations on 105 male students of Vizagapatam. Reddy and Sastry (1944) repeated estimations in 310 individuals of which 213 were students. They respectively obtained the averages of 3.156 and 2.985 litres. They got best correlation between vital capacity and sitting height. Lundgren, Sen Gupta and Saha (1953) studying the lung volumes and maximal breathing capacity among Indian men with sedentary occupations obtained the average of 3.57 litres and it was 6 per cent smaller in recumbent than in standing body position. No difference was found by them in comparisons of the vital capacity during the comfortable winter season and the hot pre-monsoon season. It is evident from the above results and on the basis of the existing data that no conclusion of a racial factor can be drawn.

The vital capacity estimations were mainly carried out among the students of Bombay, Madras and Bengal and were not made in other important States and in different categories of working classes. Correlations which were worked out by different authors were also not uniform.

The aboriginal tribes of India are generally living in the hills, in the frontiers and in some isolated areas. They have generally well built bodies, possess unlimited energy and can do quite a heavy manual work. At the same time, all the tribes are not of the same physical capacity, the nutritional quality of their dietaries vary considerably and the rates of growth of their children differ strikingly. Vital

capacity test has not been hitherto made on the tribes and such studies will in one way express their comparative physical fitness.

The studies on the vital capacity of Nokte Nagas is a part of the research programme of the Department of Anthropology on the diet, nutrition, health, physical fitness, nutritional deficiencies, nutritional status and growth of children, vital capacity, basal metabolism and other dietary and nutritional problems of the tribes of India and to these investigations the author is entrusted.

THE NOKTE NAGAS

Besides the more advanced Nagas living in the Naga hills district within Assam State different sections of Nagas dwell in the two frontier districts of Tirap Frontier Division and Tuensang Frontier Division in N.E.F.A. Culturally, socially and linguistically all these sections of Nagas are quite different. The Nokte Nagas are the inhabitants of one sub-division of Tirap Frontier Division just on the border of north-western Burma, inhabited mostly by the unadministered tribes. These Nagas were once dangerous and well known for their head-hunting but now they are law-abiding people and extended full co-operation to the investigating party under the leadership of the author. These tribes were not found to be as strong and as vigorous as those of the Padam, Minyong and Galong tribes of Abor hills where the author had the opportunity to work for many years. Anthropological investigations were carried out among the Nokte Nagas during the period from March to June, 1954.

INVESTIGATIONAL PROCEDURES

Subjects.—The subjects were all healthy male adults between approximately 18 to 45 years age.

General.—The observations on the vital capacities of Nokte Nagas were made between March to June, 1954. The climate was very moist as it was raining heavily during the period. The estimations were made either in the morning between 9 to 11 and in the afternoon between 3 to 4-30. The investigation will be continued among other tribes for a comparative study.

Age.—All the subjects were adults. Approximate ages were recorded but correct ages could not be ascertained since they did not know counting and could not state real age. Therefore no correlation has been attempted in this paper between age and other physical measurements or vital capacity.

Standing height.—This was measured on a wooden platform with anthropometer graduated in millimetres.

Sitting height (stem length).—This was measured by seating the subject on a wooden chair and measuring the stem height with the anthropometer.

Weight.—The weight was recorded in pounds to the nearest half pounds and then the results were converted to kilos.

Pelidisi.—Various indices were suggested on the basis of anthropological measurements, mainly height, sitting height and weight, to determine the nutritional state. Pelidisi, originated by von Pirquet (1922, quoted by Mason, 1931), was calculated from the formula:—

$$\sqrt[3]{\frac{10 \times \text{weight (g.)}}{\text{sitting height (cm.)}}} \times 100$$

and gave ideal values of 100 for small children and 97-98 for the adults and the values below 90 and above 110 denoted respectively undernutrition and obesity. The index fitted well in the western countries for a considerable period but the Indian workers did not find correlation with vital capacity. Calculations have been

made in this paper to determine if pelidisi of Nokte Nagas is correlated with their vital capacities.

Vital capacity.—For this determination, a wet type chain compensated spirometer calibrated in c.c., having a hollow light metalled cylinder, supplied by Messrs. C. F. Palmer Ltd., London, was used. The subject was instructed to take maximum inhalation in standing position and expel out the air in the cylinder through a metallic mouthpiece and rubber tube connection with the spirometer with maximum possible effort. The maximum value of the three efforts was recorded in each case.

Statistical methods.—The results obtained by the above procedures have been tabulated and the means, standard deviations and coefficients of variation have been worked out for each measurement. Correlation coefficients have also been computed between vital capacity and each of the other measurements. This is to determine whether the relationship between vital capacity and each of the other measurements is significant and whether this relationship is linear.

RESULTS

The actual data obtained on 140 subjects are recorded in Tables I, II, III, IV and V. In these Tables correlation between vital capacity and height, sitting height, weight, body surface area and pelidisi have been shown. At the bottom of each Table vital capacity corresponding to each range of each measurement and vital capacity per sq. m. of surface area have also been incorporated. The ranges of measurements, the mean values, standard deviations and coefficients of variation are given in Table VI. The correlation coefficients and the vital capacity per unit of each measurement are shown in Table VII. A comparative study of the vital capacity in Indians, non-Indians and of the Nokte Nagas has been made in Table VIII. In figures 1 to 5 are shown the correlation between vital capacity and standing height, approximate age, sitting height, weight, body surface area and pelidisi. The age range of the subjects represents the physically fit and able bodied persons. The mean vital capacity in the subjects was 3,035 c.c. or 2,004 c.c. per sq. m. surface area.

TABLE I

Correlation between vital capacity and height, in Nokte Nagas

Vital capacity (in litres)	Height in cm.							
	Total	140-144	145-149	150-154	155-159	160-164	165-169	170-175
Total subjects	140	2	11	27	52	35	11	2
1.6-1.9	2	..	1	1
1.91-2.2	4	..	1	3
2.21-2.5	12	1	2	3	4	1	1	..
2.51-2.8	24	1	3	6	8	5	1	..
2.81-3.1	45	..	4	10	20	9	1	1
3.11-3.4	29	3	13	10	3	..
3.41-3.7	15	5	7	3	..
3.71-4.0	7	2	3	1	1
4.01-4.2	2	1	1	..
Average vital capacity (in litres) ..		2.45	2.55	2.79	3.06	3.23	3.39	3.45
Vital capacity per sq. m. (in litres)		1.89	1.85	1.93	2.01	2.05	2.04	1.94

TABLE II

Correlation between vital capacity and sitting height in Nokte Nagas

Vital capacity (in litres)	Sitting height in cm.									
	Total	73- 75	76- 78	78- 79	80- 81	82- 83	84- 85	86- 87	88- 89	90- 92
Total subjects ..	140	3	3	24	28	32	22	14	9	5
1.6-1.9 ..	2	2
1.91-2.2 ..	4	2	1	1
2.21-2.5 ..	12	..	1	5	2	2	1	1
2.51-2.8 ..	24	2	..	5	5	5	5	2
2.81-3.1 ..	45	1	..	5	11	9	8	6	3	2
3.11-3.4 ..	29	..	1	2	6	8	6	2	3	1
3.41-3.7 ..	15	..	1	1	3	5	1	2	2	..
3.71-4.0 ..	7	1	..	2	1	1	1	1
4.01-4.2 ..	2	1	1
Average vital capacity (in litres) ..		2.77	2.93	2.79	2.94	3.08	3.07	3.18	3.27	3.48
Vital capacity per sq. m. (in litres) ..		2.07	2.13	1.93	1.86	2.00	2.01	2.06	2.00	2.08

TABLE III

Correlation between vital capacity and weight in Nokte Nagas

Vital capacity (in litres)	Weight in kilos						
	Total	40.0- 44.4	44.5- 49.4	49.5- 54.4	54.5- 59.4	59.5- 64.4	64.5- 70.0
Total subjects ..	140	8	20	59	39	9	5
1.6-1.9 ..	2	2
1.91-2.2 ..	4	..	3	1
2.21-2.5 ..	12	1	2	9
2.51-2.8 ..	24	4	4	5	10	1	..
2.81-3.1 ..	45	1	6	24	13	..	1
3.11-3.4 ..	29	..	5	10	9	4	1
3.41-3.7 ..	15	8	4	2	1
3.71-4.0 ..	7	1	2	2	2
4.01-4.2 ..	2	1	1
Average vital capacity (in litres)		2.47	2.78	2.98	3.17	3.38	3.59
Vital capacity per sq. m.		1.88	1.97	1.99	2.02	2.05	2.05

TABLE IV

Correlation between vital capacity and body surface area in Nokte Nagas

Vital capacity (in litres)	Body surface area in sq. m.									
	Total	1.3- 1.34	1.35- 1.39	1.4- 1.44	1.45- 1.49	1.5- 1.54	1.55- 1.59	1.6- 1.64	1.65- 1.69	1.7- 1.75
Total subjects ..	140	9	4	19	21	38	26	14	2	7
1.6-1.9 ..	2	1	1
1.91-2.2 ..	4	2	..	2
2.21-2.5 ..	12	1	..	4	2	3	1	1
2.51-2.8 ..	24	4	1	3	3	7	5	1
2.81-3.1 ..	45	1	1	8	11	12	8	2	1	1
3.11-3.4 ..	29	..	1	2	3	8	7	7	..	1
3.41-3.7 ..	15	1	7	2	3	..	2
3.71-4.0 ..	7	1	3	3
4.01-4.2 ..	2	1	1	..
Average vital capacity (in litres) ..		2.44	2.62	2.71	3.09	3.1	3.14	3.18	3.55	3.61
Vital capacity per sq. m. (in litres) ..		1.85	2.00	1.70	2.02	2.03	2.08	1.96	2.19	2.01

TABLE V

Correlation between vital capacity and pelidisi in Nokte Nagas

Vital capacity (in litres)	Pelidisi									
	Total	83- 86	87- 89	90- 92	93- 95	96- 98	99- 101	102- 104	105- 107	108- 110
Total subjects ..	140	1	3	11	24	45	35	9	10	2
1.6-1.9 ..	2	1	1
1.91-2.2 ..	4	1	2	1
2.21-2.5 ..	12	1	2	4	4	..	1	..
2.51-2.8 ..	24	2	6	5	5	3	3	..
2.81-3.1 ..	45	1	1	5	8	12	13	3	2	1
3.11-3.4 ..	29	..	2	2	2	13	7	2	1	..
3.41-3.7 ..	15	4	5	3	1	1	1
3.71-4.0 ..	7	3	2	..	2	..
4.01-4.2 ..	2	1	1
Average vital capacity (in litres) ..		3.10	3.25	3.07	2.92	3.04	3.00	3.14	3.11	3.70
Vital capacity per sq. m. (in litres) ..		1.98	2.17	2.04	1.97	1.99	2.03	2.06	2.01	2.14

TABLE VI

*Statistical distribution of data**Number of subjects : 140. Sex : Male*

Measurement	Range	Mean	Standard deviation	Coefficient of variation per cent
Age in years	16 - 45	30.4	7.28	23.95
Standing height, cm. ..	142.5 - 170.7	157.38	5.38	3.42
Sitting height, cm. ..	72.6 - 99.1	82.59	3.74	4.53
Weight, kg. . . .	41.6 - 66.4	53.72	5.37	10.00
Surface area (Du Bois), sq. m.	1.28 - 1.81	1.514	0.031	2.05
Pelidisi (von Pirquet index)	83.2 - 109.9	98.3	4.25	4.52
Vital capacity, litres ..	2.14 - 4.21	3.035	0.4612	15.19

TABLE VII

Statistical correlation of vital capacity and other measurements

Correlation data	Correlation coefficient	Vital capacity per unit (in litres)
<i>Vital capacity and—</i>		
Standing height ..	0.702	0.0195 per cm.
Sitting height ..	0.606	0.036 per cm.
Weight ..	0.68	0.056 per kilo.
Surface area (Du Bois) ..	0.571	2.004 per sq. m.
Pelidisi ..	0.524

TABLE VIII *

Comparative vital capacity figures for Nokte Nagas, Indians and non-Indians

Country and worker	Age (years)	Vital capacity (litres)	Vital capacity (litres) Standing height cm.	Vital capacity (litres) Surface area (Du Bois) sq. m.
<i>India—</i>				
Bhatia (1929) ..	20-45	3.096	0.0185	1.96 (Bombay)
Mukherjee and Gupta (1930) ..	20-29	3.56	0.0212	2.253 (Bengal)
Krishnan and Varood (1932) ..	18-29	2.929	0.0175	1.85 (Madras)
<i>Idem</i> (1933) ..	17-26	3.05	0.0185	1.93 (Madras)
Chatterjee (1933) ..	19	2.72 (Bengal)
Calcutta University (1934) ..	17-20	3.0	0.0182	1.91 (Bengal)
De and De (1939) ..	17-23	2.721	0.0165	1.79 (Bengal)
Niyogi and Patwardhan (1939)	18-36	2.552	0.0154	1.636 (Bombay)
Telang and Bhagwat (1941) ..	18-29	2.949	0.0176	1.83 (Bombay)
Reddy (1933)	3.156	0.0191	1.95 (Vizagapatam)
Reddy and Sastry (1944) ..	17-43	2.985	0.0179	1.911 (Vizagapatam)
<i>Indian average: -</i>				
Men (from above data) ..	17-43	2.974	0.018	1.901
Women (Mason, 1932) ..	16-35	2.150 (Madras)
<i>Nokte Nagas—</i>				
(Sen Gupta, present paper) ..	16-45	3.035	0.0195	2.004 (T.F.D.)
<i>Chinese</i> (Foster, Hsieh, 1923) ..				
Adults	..	3.18	0.0195	2.02
<i>Japanese</i> (Satake, Sato, 1938) ..				
19-26	..	3.8	0.023	2.38
<i>English</i> (Average) ..				
..	..	4.342	0.0249	2.42
<i>American</i> (Average) ..				
..	..	4.547	0.0262	2.54

* In preparing this table help has been taken from Reddy and Sastry (*loc. cit.*) and Telang and Bhagwat (*loc. cit.*).

DISCUSSION AND CONCLUSION

The age range of the subjects is large covering almost all the able bodied persons engaged in manual labour. Ages shown in this paper are approximate as no definite information could be obtained from the tribe in this regard. Excepting graphical correlation (Figure 1) and statistical treatment in Table VI, no other consideration has been given to age in this paper.

In Table I and Figure 2 are shown the relationship between vital capacity and standing height. This relationship is linear. 114 (or 81.4%) of the subjects are of stature between 150 and 164 cm. and have a vital capacity of 2.79 to 3.23 litres (or 1.93 to 2.05 litres per sq. m. of body surface area). The average stature of the tribe is 157.38 cm. and the vital capacity per cm. of standing height is 0.0195 litre.

Sitting height is not correlated with the vital capacity so significantly as that of the standing height and weight. It is evident from Table II that 120 or 87.7% of the subjects have sitting height between 78 to 87 cm. and vital capacity of 2.79 to 3.18 litres. It will be seen from Figure 3 that the vital capacity more or less is in one level between 73 to 81 cm. and shows a gradual rise above that height up to 91 cm. For this reason this measurement of Nokte Nagas has no significant value in relation to vital capacity. Similar observation was also recorded by Krishnan

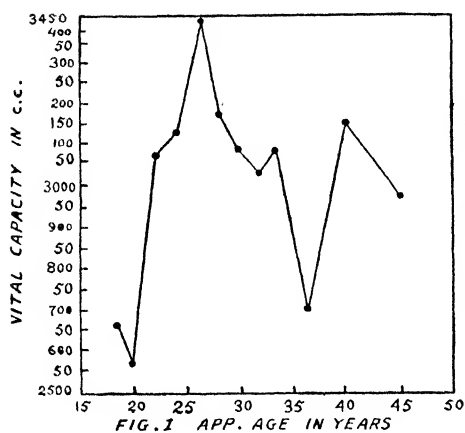


FIG. 1. APP. AGE IN YEARS

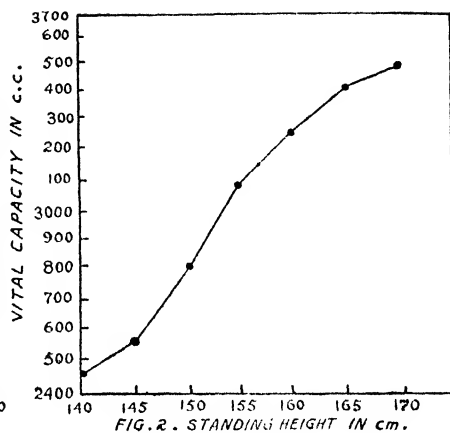


FIG. 2. STANDING HEIGHT IN cm.

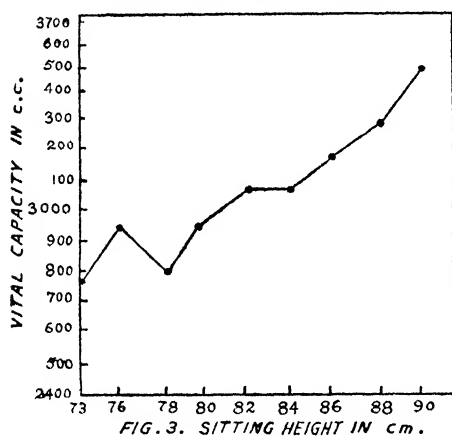


FIG. 3. SITTING HEIGHT IN cm.

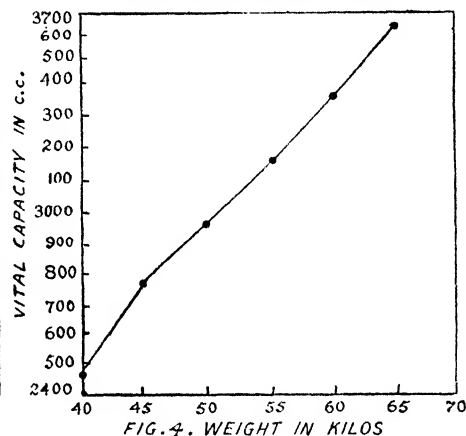


FIG. 4. WEIGHT IN KILOS

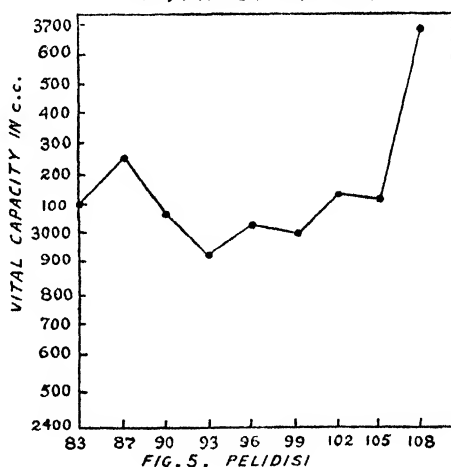


FIG. 5. PELIDISI

and Vareed (*loc. cit.*) in Vizagapatam. Telang and Bhagwat (*loc. cit.*) obtained more uniform curve. It is more variable than the standing height but much less so than those of weight and vital capacity.

A satisfactory correlation between weight in kilos and vital capacity exists and it is as significant as that between vital capacity and standing height. The linear relationship is evident from Figure 4. Table III shows that 118 or 84.3% of the subjects are of weight between 44.5 and 59.4 kilos and have the vital capacity of 2.78 to 3.17 litres (1.97 to 2.02 litres per sq. m. surface area).

Body surface area was suggested by West (*loc. cit.*) as standard for vital capacity. It is evident from Table VI that the body surface area in Nokte Nagas is least variable but it has no significant correlation with vital capacity. The average vital capacity per sq. m. of body surface is 2.004 litres. Besides the result obtained by Mukherjee and Gupta (*loc. cit.*) no other worker in India obtained such a high value.

The relationship of vital capacity with age and pelidisi in Nokte Nagas is insignificant. Such observations were also made by Telang and Bhagwat (*loc. cit.*).

The Nokte Nagas have smaller anthropometric measurements than the average Indians but their vital capacity is almost the same with that of the Indians. Comparative measurements of average Indians and Nokte Nagas are respectively shown here: standing height—166.4 and 157.4 cm., sitting height—86.8 and 82.6 cm., weight—55.4 and 53.7 kilos and body surface area (Du Bois)—1.64 and 1.51 sq. m.

The coefficient of variation ranged from 2.05 for surface area to 23.95 for approximate age. The coefficient of variation for standing height is 3.42 and then come sitting height and pelidisi. Weight and vital capacity show a greater variation, respectively 10.0 and 15.19 per cent. Telang and Bhagwat (*loc. cit.*) obtained a variation of 15.97 per cent for vital capacity.

In Nokte Nagas the most significant correlation of vital capacity is with standing height and weight and next follows the correlation with sitting height and surface area.

The tribe has an average vital capacity of 3.035 litres, which is almost the same as that of the Indian average, shown in this paper. On the basis of earlier work the Indian average was found to be 2,982 and 2,949 respectively by Telang and Bhagwat and Reddy and Sastry. The vital capacity and the ratios between vital capacity and standing height and vital capacity and surface area in Nokte Nagas are almost the same with those values in the Chinese but these are much smaller than the corresponding Japanese, English and American standards. The results prove obviously that comparatively much cooler climatic conditions in this frontier hills and much more physical exercises of the tribe have practically no influence on their vital capacity. In them also physical builds play important rôle in the variation of their vital capacity.

SUMMARY

The vital capacity of 140 adult male Nokte Nagas, inhabitant of the Tirap Frontier Division in N.E.F.A. was estimated with the help of a wet type spirometer, manufactured by C. F. Palmer Ltd., London, during March to June, 1954. Along with this, physical measurements of standing height, sitting height and weight of each subject were also taken. Body surface area (Du Bois method) and pelidisi, an index of nutrition, were also calculated from these measurements. Actual data showing the relation between vital capacity and other physical measurements are presented in five tablos. Statistical data on ranges, means, standard deviations, coefficients of variation were computed for each measurement. Coefficients of correlation between vital capacity and standing height, sitting height, weight, body surface area in sq. m. and pelidisi were worked out. The results have been compared with the average Indian, Chinese, Japanese, English and American standards. The statistical correlation between vital capacity and standing height and weight are significant. Vital capacity and other related ratios in the Nokte Nagas are identical with those of the Indians, and Chinese but these are much lower than the average Japanese, English and American standards.

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OBSERVATIONS ON THE BREEDING HABITS AND OVARIAN CYCLE IN THE INDIAN SHEATH-TAILED BAT, *TAPHOZOUS* *LONGIMANUS* (HARDWICKE)

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I. INTRODUCTION

A study of the reproductive habits of *Taphozous longimanus* was undertaken because there is no detailed account of the sex-cycle and breeding seasons of any member of the family Emballonuridae amongst the microchiroptera. Blandford (1888) observed that a female of *Taphozous longimanus* collected at Calcutta in July had a young adhering to the breast. Matthews (1941) recorded that in *Caelura afra* 'The breeding season occurs several weeks later in the year than it does in other species of bats.' Beyond such casual observations no details have been mentioned with regard to the breeding seasons of these species by the authors.

Review of literature on the reproductive phenomena of bats has been given by several authors (Duval, 1895; Hartman, 1933; Baker and Bird, 1936; Wimsatt, 1942 and Gopalakrishna, 1947). The reproductive patterns of bats so far studied can be classified into the following types:—(i) Copulation takes place in autumn and the inseminated sperms hibernate inside the genital tract of the female throughout winter and fertilize the ova in the next spring. This phenomenon has been observed by Rollinat and Trouessart (1895, 1896 and 1897) in *Vespertilio murinus* and *Rhinolophus ferrum equinum*, by Matthews (1937) in the British horse-shoe bats and by Wimsatt (1942) in *Myotis lucifugus lucifugus*. (ii) Copulation normally takes place in autumn and the inseminated sperms hibernate inside the genital tract of the female and fertilize the ova in next spring. But those females which missed copulation in autumn undergo copulation in the following spring and pregnancy follows immediately. This has been reported by Guthrie (1933) in *Myotis*

lucifugus lucifugus. (iii) Copulation takes place late in autumn and fertilization and pregnancy follow immediately and young ones are produced in the following spring. This was reported by Gopalakrishna (1950) in *Lyroderma lyra lyra*, and by Ramakrishna (1951) in *Lyroderma lyra lyra* and *Megaderma spasma*. (iv) Copulation occurs in spring and is immediately followed by fertilization and pregnancy. This has been reported by Baker and Bird (1936) in *Miniopteris australis* and by Gopalakrishna (1947) in *Scotophilus wroughltoni*.

In all the cases mentioned above there is one common feature. The species breeds only once a year in a sharply defined season and wean only one litter per year. Hence during the season when the females are pregnant they bear conceptuses of practically the same age at any given time. Production of more than one litter per year was recorded by Ramaswami (1933) in *Vesperugo leisleri* and by Matthews (1941) in *Nycteris luteola*. But these authors do not mention details of the sexual cycle. (v) A detailed account of the breeding season and female sexual cycle has been given by Wimsatt and Trapido (1952) in *Desmodus rotundus*. They record that this species behaves quite unlike other bats so far studied, in that there is no special breeding season, but it breeds throughout the year. Each female after attaining maturity brings forth at least two litters in quick succession per year.

2. MATERIAL AND METHODS

Specimens of *Taphozous longimanus* were collected in the vicinity of Nagpur and Amaravati (Madhya Pradesh). These are small bats clinging to the walls of old houses. Some specimens were also collected from the hollows of big trees. *Taphozous longimanus* is not colonial in habit, but lives either singly or in groups of three or four.

Collection of material commenced in October, 1947 and the last batch for the present study was collected in August, 1953. Excepting during the months of April and May collections were made to represent all the calendar months of the year. In all 135 females and 50 males were collected.

Specimens captured or shot by air-gun were weighed immediately. The genitalia of the females were fixed in alcoholic Bouin's fluid. In cases of some pregnant specimens other fixatives like Rassman's fluid, neutral formalin and Carnoy's fluid without acetic acid were also employed for future cytochemical study. Uteri with advanced conceptuses were slit open to facilitate proper fixation. After the usual method of dehydration and embedding sections were cut 10μ thick and stained with Delafield or Ehrlich's haematoxylin and counterstained with eosin.

The collection diary drawn from field record is appended. A summary of the collection diary with relevant details is also given for easy reference. (See Appendix and Table I.)

3. OBSERVATIONS AND DISCUSSION

(a) Breeding seasons.

The collection diary reveals several interesting features which are rather unusual amongst the insectivorous bats. Pregnant females were found in all the months of the year. The absence of collections in April and May does not seriously affect this conclusion because in June females in advanced pregnancy were found. In the record of the collections for each month as well as amongst a group of females collected at several individual collections, females are found at different stages of growth and sexual activity such as, females in advanced pregnancy, females in early pregnancy, females in full lactation carrying young at the breast and also non-pregnant females.

It is thus obvious that there is no restricted breeding season for *Taphozous longimanus*, and hence this species differs from most of the insectivorous bats so

far studied which have a sharply defined breeding season and hence show pregnancy during only certain months of the year.

In *Taphozous longimanus* there is ample evidence to show that each female becomes pregnant more than once in a year and most probably there is continuous breeding with pregnancies following in quick succession. These conclusions are borne out by the following observations:—32 females collected during different months of the year were in full lactation as evidenced by the presence of a young adhering to the breast or by the exudation of milk when the nipples were squeezed. Of these 16 showed on dissection that they were pregnant also. In 5 of these cases the ovary of the non-pregnant side showed a degenerating corpus luteum of the previous pregnancy (Pl. V, fig. 11). This shows that pregnancies follow one another in quick succession. It is not possible to state with the help of the material at my disposal, how soon after parturition, does the next oestrous cycle start. This period should obviously be very short since the corpus luteum of the previous pregnancy has not undergone complete resorption when the next pregnancy has started.

Another fact which supports this conclusion is that out of 135 females collected during six years, 100 specimens showed either pregnancy or lactation or both, leaving only 16 which were neither pregnant nor in lactation; and 19 which were decidedly immature since they were very small and were attached to their mothers' breasts. Microscopic examination of the ovaries of specimens weighing 26.4 gms. and less, revealed that they were similar to the juvenile females. But the ovaries of specimens weighing more than 26.4 gms. had vesicular graafian follicles and therefore could be considered as adult specimens. The lowest body weight of a pregnant female in my collection is 31.6 gms. (the uterus in this specimen had an early implanted blastocyst), and excepting two specimens every female which weighed 31.6 gms. and over was either pregnant or in lactation. The two exceptional females weighed 32.8 gms. and 32.4 gms. respectively and their ovaries (Pl. IV, fig. 4 and Pl. V, fig. 9) contained large vesicular graafian follicles suggestive of approaching pro-oestrus. The nipples of the mammary glands of these specimens were small. It is very probable that these specimens were on the threshold of their first oestrous cycle. The foregoing arguments suggests that all the non-pregnant females in my collection were also non-parous without having become pregnant even once. This percentage is so small in the total population that it could only mean that after the attainment of sexual maturity the females breed continuously without there being any pronounced anoestrus period between successive pregnancies.

How many litters does a female produce per year cannot be determined because I have no criterion to decide the period of gestation.

Thus *Taphozous longimanus* differs from most of the insectivorous bats and resembles only *Desmodus rotundus* (Wimsatt and Trapido, 1952). It is noteworthy that the other species of bats which are reported to bring forth more than one litter per year such as *Vesperugo leisleri* (Ramaswami, 1933), *Nycteris luteola* (Matthews, 1941) and *Desmodus rotundus* (Wimsatt and Trapido, 1952) are tropical species. The occurrence of continuous breeding in *Taphozous longimanus* inhabiting a region like Nagpur where the summer is very hot, the rainy season is confined to the months of June, July, August and part of September and the winter is fairly cold is interesting because the seasonal changes in the climatic conditions are very marked.

(b) Sex-ratio.

There seems to be a very abnormal sex-ratio in *Taphozous longimanus*. During six years of random collection 135 females and 50 males were collected. The occurrence of an abnormal sex-ratio, with females predominating, has been observed

in many other species of insectivorous bats by earlier workers (Blandford, 1888; Phillips, 1935; Baker and Bird, 1936; Wimsatt, 1945; Abdulali, 1948; Gopalakrishna, 1947 and 1950; Ramakrishna, 1951). In these species worked out by these authors there is a sharply defined breeding season and therefore there is a possibility that the males and the females live in separate colonies excepting at the time of breeding. It may, therefore, be difficult to determine the actual sex-ratio in these species. But this difficulty is not encountered in *Taphozous longimanus* as it breeds throughout the year and is not colonial in habit. Further, the collection data shows that this abnormality in sex-ratio is found in all the months of the year. Even the immature ones collected from the breasts of the mothers show this disparity in numbers—19 females to 10 males. Hence the proportion of females to males as is indicated by the collection record should be very nearly the natural sex-ratio in this species.

(c) *General plan of the female reproductive organs.*

As in most insectivorous bats (excepting the members of the family Phyllostomidae) the female reproductive organs consist of a pair of ovaries, a bicornuate uterus and a non-septate vagina. The ovaries are oval in shape and are enclosed in a complete ovarian capsule. The fallopian tube arises from the median aspect of the ovarian capsule, takes a circuitous course over the capsule and joins the respective uterine cornu. The uterine cornua are equally developed and meet posteriorly at an acute angle forming a 'V' shaped structure. The uterine cervix projects a short distance into the vagina which opens to the outside on a slight elevation.

(d) *Number of embryos in a litter.*

Though the uterus is bicornuate and equally developed only one young is produced in each litter, and the embryo may be either in the right horn or in the left horn of the uterus. In 88 specimens in which the uterus unmistakably showed either pregnancy or post-partum condition, 39 had it in the right horn and 49 in the left. Further in the cases of pregnant females whose ovaries were sectionised, the corpus luteum occurred in the ovary of the same side in which the uterine horn had pregnancy. In 5 cases of females which showed post-partum pregnancy the ovary of the non-pregnant side had a degenerate corpus luteum of the previous pregnancy. This taken along with the fact that there is quick succession of pregnancies indicates that ovulation alternates between the two ovaries and pregnancy alternates between the two horns of the uterus in successive pregnancies.

In most microchiroptera only one young is produced in each litter. Only in a few species more than one is brought forth in each litter (Ramaswami, 1933; Wimsatt, 1945; Gopalakrishna, 1947; Uchida, 1950). Further in several monotokous bats there is a tendency for the genitalia to become physiologically asymmetrical and only one side to become functional, and in most cases the foetus is borne in the right horn (Jones, 1917; Guthrie, 1933; Baker and Bird, 1936; Matthews, 1937; Wimsatt, 1945). In extreme cases like *Rhinolophus hipposiderus minutus* (Matthews, 1937) the left ovary does not even produce mature ova and shows signs of atrophy. Though *Taphozous longimanus* consistently brings forth a single young each time there is no physiological asymmetry, but presumably there is an alternation of the two sides of the genitalia in successive pregnancies.

While three or four follicles undergo development simultaneously to full size, only one undergoes rupture in *Taphozous longimanus*. I have not got a single specimen which showed more than one corpus luteum in the ovary. In several monotokous bats more than one ovum may be shed (Wimsatt, 1945; Wimsatt and Trapido, 1952).

(e) *Microscopic examination of the ovaries.*

(i) *Juvenile ovary.*—The following description refers to the ovaries of specimens which were attached to the breasts of their mothers. The body weights range between 10.6 gms. to 18.6 gms. There is not much difference in the structure of the ovaries of the different specimens or between the ovaries of the two sides of the same specimen (Pl. IV, fig. 1).

The germinal epithelium consists of cuboidal cells, each with a large spherical nucleus centrally placed in the cell (Pl. IV, fig. 2). Here and there some cells of the germinal epithelium are larger than the rest of the cells of the germinal epithelium and they bulge inwards. These are unmistakably the mother cells of the oocytes and they migrate inwards towards the deeper regions of the ovary during further development. As the oocyte migrates inwards it becomes surrounded by a circlet of flattened cells—the satellite cells of the primordial follicle. The peripheral part of the ovary contains a large number of such primordial follicles (Pl. IV, fig. 2).

As the follicles increase in size the follicle cells become at first cuboidal and later columnar with the nuclei occupying the distal poles of the cells thus leaving a hyaline region of cytoplasm immediately surrounding the ovum. There is also a progressive increase in the number of the follicle cells.

The tunica albuginea is imperfectly developed and there is no clear distinction between the cortical and medullary part of the ovary.

In the deeper regions of the ovary there are a number of unilaminar follicles (Pl. IV, fig. 3). The oocyte nucleus becomes eccentric by the time the unilaminar follicle reaches its full size. The follicles in the juvenile specimens do not develop beyond the unilaminar stage because follicular atresia set in before the bilaminar stage is reached. The onset of follicular atresia is indicated by the oocyte losing its spherical shape and becoming irregular. The nucleus becomes highly pyknotic and finally the oocyte undergoes degeneration. Atresia of the follicle cells follow the degeneration of the oocyte so that for a short time while the oocyte is undergoing degeneration there is an actual increase in the size of the follicle and mitotic division of the follicle cells continues even though the oocyte shows distinct signs of degeneration.

(ii) *Growth changes in the ovary.*—The histological details of the ovary undergo but little change until the specimen reaches a body weight of 26.4 gms., while the ovaries of specimens weighing more than 26.4 gms. show a distinct advance over the ovaries of the juvenile ones (Pl. IV, fig. 6). The germinal epithelium is characterized by the occurrence of more epithelial nodules—the fore-runners of oocytes—indicating an increase of the ovogenetic potentiality of the germinal epithelium. The ovarian medulla contains follicles in advanced stages of development (Pl. IV, fig. 5).

The development of the follicle follows the same pattern as in other mammals. During the early stages of growth there is a simultaneous increase of the oocyte and the follicle as a whole, but after the oocyte reaches its maximum size only the follicle increases, first by the proliferation of the cells and later by the formation and growth of the antrum.

The fully formed graafian follicle (Pl. V, fig. 7) shows certain interesting details. The theca folliculi consists of 4 to 6 layers of closely packed fusiform cells. A clear distinction between the theca externa and the theca interna cannot be made out. Small capillaries are seen in this layer. The theca is predominantly cellular and the general fibrous appearance is due to the fact that the cells of the theca are fusiform and their nuclei spindle shaped. The granulosa layer has a distinct basement membrane and consists of 8 to 10 layers of cells with spherical nuclei. The oocyte is placed eccentrically and is attached to the granulosa layer. A definite conical cumulus as is noticed in many mammals is not present—the granulosa layer being as thick in the region of the attachment of the ovum as in the rest of the

follicle. A very interesting feature of the graafian follicle of *Taphozous longimanus* is that there is a single layer of cells surrounding the oocyte. The antrum is filled with liquor folliculi.

The fully formed graafian follicle of *Taphozous longimanus* has, therefore, certain unique characters. The oocyte of *Myotis lucifugus lucifugus* (Wimsatt, 1944; and Guthrie and Jeffers, 1938) is supported within the follicle by a large number of polygonal cells extending from the region of the discus to the granulosa. This is also the case in *Scotophilus wroughtoni* (Gopalakrishna, 1949). Obviously it seems that there are differences in the histological details of the graafian follicle in particular and the ovaries in general in the different species of bats. Probably a careful examination of the ovaries might reveal that there are fundamental differences between species.

Follicular atresia is common in the adult ovaries and most often it sets in when the follicle reaches the multilaminar condition. Some follicles undergo degeneration at later stages of development of the follicle.

(iii) *Changes in the ovaries of the pregnant female.*—The most striking feature of the ovary of the pregnant side in a pregnant specimen is the presence of the corpus luteum. In a specimen having an early implanted blastocyst the corpus luteum occupies about half of the ovary (Pl. V, fig. 8). The central cavity of the corpus luteum has disappeared and its place is occupied by large vacuolated cells. By the time the embryo reaches the limb-bud stage of development the corpus luteum has grown enormously and occupies practically the entire ovary. Centripetally arranged fibrous strands are present in the corpus luteum (Pl. V, fig. 10).

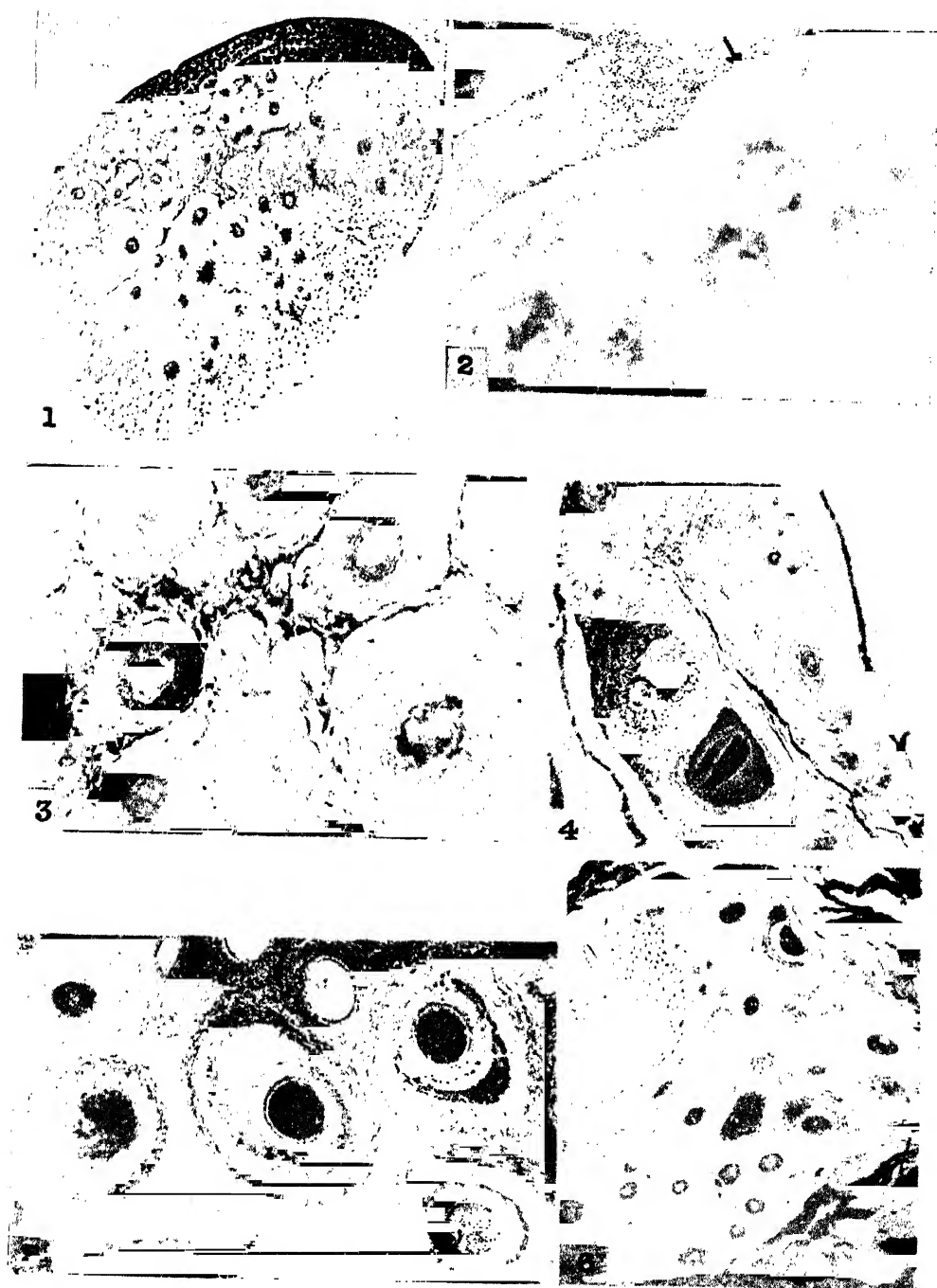
The histology of the ovary of the non-pregnant side during pregnancy does not seem to have attracted the attention of workers so far. During very early pregnancy the ovary of the non-pregnant side of 5 specimens contained a degenerate corpus luteum of the previous pregnancy (Pl. V, fig. 11). As pregnancy advances the histological details of the ovary on the non-pregnant side also shows change. Follicular development continues as in a non-pregnant female excepting that the follicle undergoes degeneration at the vesicular stage. In a transverse section of the ovary of the non-pregnant side of a pregnant specimen a number of vesicular follicles can be seen. It is interesting to note that the ovary of the non-pregnant horn does not seem to remain quiescent but shows all stages of activity except the actual development of the graafian follicle to its maximum size. In a specimen showing advanced pregnancy the ovary of the non-pregnant side (Pl. V, fig. 12) contained three well developed graafian follicles with antra. This is an added circumstantial evidence to support the conclusion that there is continuous breeding and that there is a functional alteration of the two sides of the genitalia in successive pregnancies.

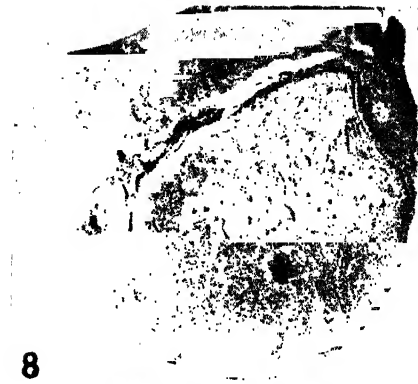
4. SUMMARY

1. The paper embodies observations on the breeding habits and the ovarian cycle in *Taphozous longimanus*.
2. *Taphozous longimanus* breeds all round the year and there is a quick succession of pregnancies.
3. There is a very abnormal sex-ratio with females predominating over males in this species.
4. A single young is brought forth in each litter, and the pregnancy alternates between the two horns of the uterus.
5. Ovulation occurs in the ovary of the same side in which there is pregnancy.
6. The fully formed graafian follicle differs from that of most other bats in having a single layer of cells surrounding the ovum.
7. The ovary of the non-pregnant side of a pregnant specimen shows continued activity.

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7. APPENDIX

Collection diary of Taphozons longimanus (Hardwicke)

Date of collection	Sp. No.	Body weight in gms.	Sex	Particulars
<i>January:</i>				
16-1-1952	.. A*	..	Female ..	Lactating, with young at breast (C). Right horn of uterus shows pregnancy again.
"	.. B	..	" ..	Lactating; young lost. Post-partum uterus right horn.
"	.. C	..	" ..	Young from breast of 'A'.
"	.. D	..	Male ..	Adult male.
"	.. E	..	" ..	Adult male.
18-1-1949	.. 36	46.2	Female ..	Advanced pregnancy in right horn.
"	.. 37	32.8	" ..	Non-pregnant.
"	.. 38	36.4	Male ..	Adult.
28-1-1950	.. 64	42.0	Female ..	Advanced pregnancy in right horn.
"	.. 65	43.0	" ..	Early pregnancy in left horn.
<i>February:</i>				
6-2-1953	.. RR	14.4	Female ..	Young; mother escaped.
16-2-1948	.. 14	26.0	" ..	Non-pregnant.
16-2-1953	.. 139	32.8	" ..	Mid-pregnancy in right horn.
"	.. 140	32.6	" ..	Early pregnancy in left horn.
25-2-1953	.. 141	36.4	" ..	Early pregnancy in right horn.
"	.. 142	33.8	Male ..	Adult.
"	.. 143	37.6	" ..	Adult.
26-2-1953	.. 144*	37.4	Female ..	Lactating; young lost; again pregnant in right horn.
"	.. 145	36.8	Male ..	Adult.
28-2-1953	.. ZZ	30.2	Female ..	Non-pregnant.
"	.. MM	12.6	" ..	Young; mother escaped.
"	.. KK	36.4	" ..	Early pregnancy in right horn.
"	.. SS	38.2	Male ..	Adult.
"	.. TT	36.0	" ..	Adult.
<i>March:</i>				
3-3-1948	.. 15*	34.3	Female ..	Lactating; early pregnancy in right horn.
"	.. 16	36.2	" ..	Early pregnancy in right horn.
"	.. 17	30.5	Male ..	Adult.
"	.. 18	42.4	Female ..	Advanced pregnancy in left horn.
3-3-1953	.. 150	34.5	" ..	Early pregnancy in left horn.
"	.. 151	38.4	" ..	Early pregnancy in left horn.
"	.. 152	35.8	Male ..	Adult.
"	.. 153	41.2	" ..	Adult.
"	.. 154	16.1	" ..	Young; mother escaped.
10-3-1953	.. 155	43.5	Female ..	Advanced pregnancy in right horn.
13-3-1949	.. 39*	32.5	Female ..	Mother lactating with a young (44) in the breast; early pregnancy in right horn.
"	.. 40	36.2	" ..	Early pregnancy in left horn.
"	.. 41	41.0	" ..	Advanced pregnancy in left horn.
"	.. 42	31.6	" ..	Early pregnancy in left horn.
"	.. 43	28.2	" ..	Non-pregnant.
"	.. 44	16.2	" ..	Young from breast of Sp. No. 39.
"	.. 45	36.0	" ..	Lactating, right horn post-partum. Young lost.

APPENDIX—Contd.

Collection diary of Taphozous longimanus (Hardwicke)

Date of collection	Sp. No.	Body weight in gms.	Sex	Particulars
<i>April: No collections.</i>				
<i>May: No collections.</i>				
<i>June:</i>				
26-6-1948	.. 19	38.4	Female ..	Advanced pregnancy in right horn.
"	.. 20	32.0	Male ..	Adult.
26-6-1953	.. AA	30.5	Female ..	Non-pregnant.
"	.. BB	12.6	Male ..	Young; mother escaped.
"	.. CC	13.2	Female ..	Young from the breast of Sp. No. DD.
"	.. DD	36.5	" ..	Lactating with young (CC) at breast.
"	.. EE	39.0	" ..	Early pregnancy in right horn.
"	.. FF	42.4	" ..	Advanced pregnancy in left horn.
"	.. GG	40.5	" ..	Lactating; post-partum on right side.
"	.. HH*	38.2	" ..	Lactating; young lost, again pregnancy in left horn.
"	.. II	11.5	" ..	Young; mother escaped.
<i>July:</i>				
6-7-1953	.. J(1)	31.2	Female ..	Non-pregnant.
"	.. J(2)	44.4	" ..	Lactating with young at breast J(3).
"	.. J(3)	10.6	" ..	Young from the breast of Sp. No. J(2).
"	.. J(4)	29.2	" ..	Non-pregnant.
"	.. J(5)	32.4	" ..	Non-pregnant.
11-7-1948	.. 21	41.4	" ..	Advanced pregnancy in right horn.
"	.. 22	28.2	Male ..	Adult.
28-7-1948	.. 23	24.2	" ..	Adult.
<i>August:</i>				
16-8-1948	.. 24	31.0	Male ..	Adult.
18-8-1952	.. 66	44.2	Female ..	Advanced pregnancy in right horn.
18-8-1953	.. Au(1)	32.4	Male ..	Adult.
21-8-1952	.. 67	13.2	Female ..	Young; mother escaped.
22-8-1952	.. 68	42.5	" ..	Advanced pregnancy in left horn.
"	.. 69	38.4	" ..	Advanced pregnancy in right horn.
"	.. 70	41.4	" ..	Advanced pregnancy in left horn.
"	.. 71	39.5	" ..	Advanced pregnancy in left horn.
"	.. 72	31.8	" ..	Early pregnancy in left horn.
"	.. 73	39.2	" ..	Lactating with young at breast.
"	.. 74	13.5	Male ..	Young from breast of Sp. No. 73.
29-8-1952	.. 75	..	Female ..	Early pregnancy in left horn.
30-8-1952	.. 76	40.5	" ..	Advanced pregnancy in right horn.
"	.. 77	38.0	" ..	Advanced pregnancy in right horn.
"	.. 78	41.2	" ..	Advanced pregnancy in right horn.
"	.. 79*	38.4	" ..	Lactating with young (83); again pregnant in right horn; early pregnancy.
"	.. 80	38.2	" ..	Advanced pregnancy in right horn.
"	.. 81	36.5	" ..	Early pregnancy in left horn.
"	.. 82	36.5	" ..	Mid-pregnancy in left horn.
"	.. 83	14.2	" ..	Young female from breast of Sp. No. 79.
"	.. 84	38.0	" ..	Lactating with young in breast; young lost.
"	.. 85	40.0	" ..	Advanced pregnancy in right horn.
"	.. 86	43.3	" ..	Advanced pregnancy in right horn.

Collection diary of Taphozous longimanus (Hardwicke)

APPENDIX—Contd.

Date of collection	Sp. No.	Body weight in gms.	Sex	Particulars
<i>September:</i>				
5-9-1952	87	38.2	Female	Advanced pregnancy in right horn.
"	88	36.2	"	Early pregnancy in left horn.
"	89	44.5	"	Advanced pregnancy in left horn.
"	90	41.4	"	Advanced pregnancy in right horn.
"	91*	35.6	"	Lactating female with young (91a) in the breast. Again pregnancy in left horn.
"	91a	12.8	"	Young from breast of No. 91.
"	92	14.2	"	Young female; mother escaped.
"	93	38.0	Male	Adult.
"	94	34.2	"	Adult.
"	95	26.2	"	Adult.
"	96	15.2	"	Young from breast of No. 97.
"	97*	38.5	Female	Lactating with young in the breast (Sp. 96); again early pregnancy in left horn.
6-9-1948	25	..	Female	Early pregnancy in right horn.
"	26*	..	"	Lactating female; again pregnancy in left horn.
"	27	..	"	Advanced pregnancy in right horn.
"	28	..	"	Lactating with young at breast; young lost.
"	29	..	Male	Adult.
"	30	..	"	Adult.
20-9-1952	98	..	Female	Advanced pregnancy in right horn.
"	99*	..	"	Lactating mother. Early pregnancy in right horn.
"	100	..	"	Advanced pregnancy in left horn.
"	101	..	"	Lactating with young (104) at breast.
"	102	..	"	Non-pregnant.
"	103	..	"	Lactating with young at breast (108).
"	104	..	"	Young female from Sp. No. 101.
"	105	..	Male	Adult.
"	106	..	"	Adult.
"	107	..	"	Adult.
"	108	..	"	Young from the breast of Sp. No. 103.
"	109	..	Female	Young; mother escaped.
22-9-1949	46	31.6	"	Early pregnancy in right horn.
"	47	36.2	"	Early pregnancy in right horn.
"	48	35.0	"	Lactating with young in breast; young lost
"	49	32.0	Male	Adult.
29-9-1952	110	41.5	Female	Advanced pregnancy in right horn.
"	111	36.0	Male	Adult.
"	112	33.0	"	Adult.
"	113	26.5	"	Adult.
"	114	11.6	Female	Young female; mother escaped.
<i>October:</i>				
5-10-1952	115	..	Female	Advanced pregnancy in left horn.
"	116	..	"	Early pregnancy in left horn.
"	117	..	"	Lactating; right horn post-partum.
"	118	..	Male	Adult.
"	119	..	"	Adult.
20-10-1952	120	13.4	"	Young from breast of Sp. No. 122.

*(Collection diary of Taphozoms longimanus (Hardwicke))*APPENDIX- *Contd.*

Date of collection	Sp. No.	Body weight in gms.	Sex	Particulars
<i>October:—(Contd.)</i>				
20-10-1952 ..	121	30.3	Female ..	Non-pregnant.
" ..	122*	34.0	" ..	Lactating with young (120); again pregnancy in right horn.
" ..	123	40.5	" ..	Advanced pregnancy in left horn.
" ..	124	39.2	" ..	Advanced pregnancy in left horn.
" ..	125	36.2	" ..	Advanced pregnancy in right horn.
" ..	126	38.4	" ..	Mid-pregnancy in left horn.
21-10-1947 ..	1	41.2	" ..	Very late pregnancy in left horn.
" ..	2	36.2	" ..	Early pregnancy in left horn.
" ..	3	38.0	" ..	Early pregnancy in left horn.
" ..	4	36.0	" ..	Lactating female; right horn post-partum.
" ..	5	38.0	" ..	Lactating; right horn post-partum.
" ..	6	34.0	" ..	Non-pregnant.
" ..	7	28.0	Male ..	Adult.
29-10-1949 ..	50	34.6	Female ..	Early pregnancy in right horn.
" ..	51*	39.2	" ..	Lactating with young in breast (56); again early pregnancy in left horn.
" ..	52	36.0	" ..	Lactating; left horn post-partum. Young lost.
" ..	53	34.0	" ..	Lactating; left horn post-partum.
" ..	54	28.0	" ..	Non-pregnant.
" ..	55	31.0	Male ..	Adult.
" ..	56	12.0	" ..	Young from Sp. No. 51.
30-10-1949 ..	57*	36.2	Female ..	Lactating with young (60); again early pregnancy in right horn.
" ..	58	43.2	" ..	Advanced pregnancy in left horn.
" ..	59	42.3	" ..	Mid-pregnancy in right horn.
" ..	60	10.8	" ..	Young from breast of Sp. No. 57.
" ..	61	18.6	" ..	Young; mother escaped.
<i>November:</i>				
8-11-1948 ..	31	31.0	Female ..	Non-pregnant.
" ..	32	26.4	" ..	Non-pregnant.
" ..	33	32.6	Male ..	Adult.
10-11-1947 ..	8	46.1	Female ..	Advanced pregnancy in left horn.
" ..	9	40.2	" ..	Early pregnancy in right horn.
" ..	10	37.2	" ..	Early pregnancy in left horn.
" ..	11	36.0	" ..	Lactating with young; young lost. Post-partum horn on left side.
" ..	12	32.0	Male ..	Adult.
" ..	13	29.2	Female ..	Non-pregnant.
12-11-1952 ..	127*	.	" ..	Lactating with young (130); again early pregnancy in left horn.
" ..	128	..	" ..	Lactating female, young lost.
" ..	129	..	" ..	Lactating female, left horn post-partum young lost.
" ..	130	..	" ..	Young from breast of Sp. No. 127.
" ..	131	..	" ..	Non-pregnant.
" ..	132	..	Male ..	Adult.
24-11-1949 ..	62	44.6	Female ..	Advanced pregnancy in right horn.
" ..	63	12.3	" ..	Young female. Mother escaped.
28-11-1952 ..	MM	..	Male ..	Adult.
" ..	WW	..	" ..	Adult.

Collection diary of Taphozous longimanus (Hardwicke)

APPENDIX—Contd.

Date of collection	Sp. No.	Body weight in gms.	Sex	Particulars
<i>December:</i>				
5-12-1948 ..	34	46.5	Female	Advanced pregnancy in right horn.
" ..	35	37.2	Male	Adult.
8-12-1952 ..	133*	..	Female	Lactating with young at breast; again early pregnancy in right horn.
" ..	134	..	"	Early pregnancy in left horn.
" ..	135*	..	"	Lactating with young in breast; again early pregnancy in right horn.
" ..	136	..	"	Young female from breast of Sp. No. 133.
" ..	137	..	Male	Adult.
" ..	138	..	"	Young from the breast of Sp. No. 135.
12-12-1952 ..	De(i)	36.0	Female	Lactating female.
" ..	De(ii)	37.5	"	Lactating female.
" ..	De(iii)	38.0	Male	Adult.
" ..	De(iv)	24.2	"	Adult.
" ..	De(v)	37.0	"	Adult.

Foot note:—

The word 'Adult' has been used in the diary to denote those specimens which were not attached to the breasts of the mothers, as against the word 'Young' which were attached to the breasts of the mothers.

Specimens marked with an asterisk were lactating females which showed on dissection that one of the uterine horns had again become pregnant.

'Non-pregnant female' denotes females which were free and did not show either pregnancy or lactation.

TABLE I
Summary of collection diary

Month	Females				Males			Total
	Pregnant		Non-pregnant		Immature	Adult	Immature	
	Lact.	Non-lact.	Lact.	Non-lact.				
January ..	1	3	1	1	1	3	..	10
February ..	1	4	0	2	2	5	..	14
March ..	2	8	1	1	1	3	1	17
April ..	No Collections							
May ..	No Collections							
June ..	1	3	2	1	2	1	1	11
July	1	1	3	1	2	..	8
August ..	1	15	2	..	2	2	1	23
September ..	4	11	4	1	5	12	2	39
October ..	3	12	5	3	2	4	2	31
November ..	1	4	3	4	2	3	2	19
December ..	2	2	2	..	1	5	1	13
TOTAL ..	16	63	21	16	19	40	10	185

8. EXPLANATION OF PLATES

(All figures are microphotographs)

- FIG. 1. Section of the ovary of a juvenile specimen showing the presence of a large number of primordial follicles in peripheral part of the ovary. The central part of the ovary contains a number of unilaminar follicles. $\times 55$.
- .. 2. Enlarged part of the surface of the juvenile ovary. One of the cells in the germinal epithelium is large (arrow) and this is the forerunner of the oocyte. Below the surface are found a few of the primordial follicles with flattened satellite cells. $\times 650$.
- .. 3. Central part of the juvenile ovary. $\times 240$.
- .. 4. Section of the left ovary of a non-parous specimen in its first oestrous cycle. $\times 40$.
- .. 5. Central part of the ovary of a specimen in early pro-oestrus. $\times 110$.
- .. 6. Ovary of specimen in early pro-oestrus (entire). $\times 40$.
- .. 7. Fully formed graafian follicle. $\times 120$.
- .. 8. Ovary with corpus luteum in a specimen which had a blastocyst in the uterus of the same horn. $\times 25$.
- .. 9. Right ovary of the specimen whose left ovary is shown in fig. 4. $\times 40$.
- .. 10. Ovary with a fully developed corpus luteum in a specimen showing advanced pregnancy. $\times 40$.
- .. 11. Ovary of the non-pregnant side of a specimen showing early pregnancy in the opposite horn of the uterus. Note the degenerating corpus luteum. $\times 35$.
- .. 12. Ovary of the non-pregnant side of a specimen which had advanced pregnancy in the opposite uterine horn. $\times 50$.

Issued August 8, 1955.

CHROMATOGRAPHIC STUDIES ON THE AMINO ACID METABOLISM OF HEALTHY AND DISEASED LEAVES OF *CROTON SPARSIFLORUS*, MORONG

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(Received December 11, 1954)

INTRODUCTION

The need of accurate knowledge with regard to diverse metabolites of proteins and carbohydrates in diseased and healthy plants, has been acutely felt. Recent researches by Consden, Gordon and Martin (1944) and others have opened up new vistas for the plant physiologists. Very few workers have tried the horizontal migration method of chromatography in the study of plant physiology. Benson *et al.* (1950), Steward *et al.* (1954) and Sen and Burma (1953), however, have done substantial work using two-dimensional chromatography.

Giri (1951) used the circular paper chromatography employing single wick in the centre for the migration of the solvent. Ganguli (1954) developed an improved method by perforating his filter papers and thus separating the sample solutions from mixing up. We have tried both the methods but we could not get satisfactory picture of the chromatogram because the arcs of the adjoining sectors sometimes overlapped. We feel that the slight variation of our method described in this paper gives a considerably improved chromatogram; the *R_f* values come out consistent and the amino acid bands are clear and tailless.

The work deals with the study of water-soluble amino acids and those obtained by hydrolyzing the protein content of both healthy and diseased *Croton sparsiflorus* leaves.

MATERIALS AND METHODS

(a) *Materials*.—As noted above leaves (of the same age) of *Croton sparsiflorus*, growing in wild condition, were selected for experimentation. Being a common weed it grows in fair abundance. Often the leaves have been frequently found to be infected with 'yellow-mosaic' which caused stunting of the plant and slight curling up of the leaves. It was decided to study the changes in the protein-metabolism as a result of such an infection.

(b) *Method*.—At the initial stages, Giri's method was tried. To overcome the overlapping of some of the bands, the following method was evolved, which has been used throughout the experiment. A circular piece, of diam. 40 cm. of Whatman No. 1 filter paper, was cut out. Two small circles of radii 4 cm. and 3.5 cm. were drawn from the centre. Round the periphery of the small circle 16 perforations were made. From the outer circle sixteen radiating fins 2 mm. \times 15 cm. were made at equal distances. These were then clipped off to separate each radial sector, comprising in all 16 equal and separated sectors (see Fig. 1). A drop of liquid of .002 ml. was then gently placed on to the area marked for the purpose on the circle of diameter 4 cm. A single wick of size 2 cm. \times 4 cm. was rolled tightly and inserted in the centre for the solvent to rise. The filter paper being rested on a petri dish of 25 cm. diameter. The whole apparatus was then covered with a trough serving as a cover for the saturation chamber (Fig. 2). This

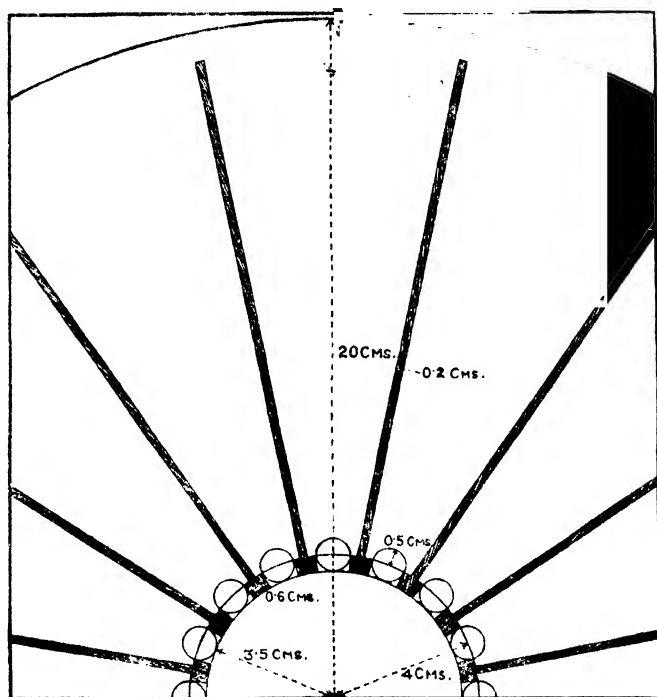


FIG. 1. Diagrammatic representation of a portion of Whatman No. 1 filter paper—40 cm. (diameter). The shaded areas are cut-off. The arrows pointing towards the centre mark the position of the wick.

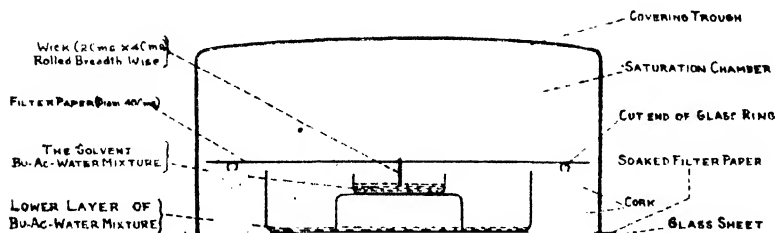


FIG. 2. Diagrammatic representation of the Chromatographic apparatus.

simple method has given quite satisfactory results and has shown distinct improvement in the clarity of the chromatogram by preventing the circular spread of the advancing liquid.

The solvent : *n.* butanol 100 c.c., glacial acetic acid 25 c.c., water 125 c.c., was used. The experiment was run for 20 hours at room temperature. Thereafter the paper was removed from the chamber, solvent front marked and the paper kept for drying. For the development of the chromatogram 0.1% nin-hydrin (in acetone) was sprayed and the chromatogram left at room temperature for 4 hours and then kept in the oven for 10 minutes at 75°C.

EXPERIMENTS

8 gms. of leaves of each type (healthy and diseased) were plucked, weighed and dropped in distilled water, which was maintained at 60°C. to kill the enzymes. The material was crushed with its water and filtered. In this filtrate (A) tannins were precipitated and removed by lead acetate (in excess). The lead being precipitated by sodium oxalate in cold. 0.1 c.c. of chloroform was added to remove the pigments and the clear filtrate was then concentrated to 20 c.c. 5 c.c. of this was then transferred to centrifuge-tubes. The solutions were centrifuged at 2,500 revolutions per minute and the clear supernatant liquid was then transferred to 50 c.c. conical flasks. The residue being quantitatively taken in 100 c.c. conical flasks with cotton plugs, 25 c.c. of 6*N* HCl was then added and autoclaved for 2 hours at 15 lb. pressure to hydrolyze the protein content completely. The filtrate after autoclaving and filtering the content was made to 40 c.c. 0.002 ml. of each sample solution was then spotted, for the identification of amino acids, by a special micropipette.

The drops of the various solutions were kept at their respective marked places as given in the following chart (*see* Plate VI):

B, H and N .. Water-soluble extract of healthy leaves.

C, I and O .. Water-soluble extract of diseased leaves.

E and K .. Hydrolysate of healthy leaves.

F and L .. Hydrolysate of diseased leaves.

** Known amino acids (for reference)*

A .. Leucine, arginine and cystine.
D .. Isoleucine, and glycine.
G .. D-L valine, D-L tyrosine, and histidine.
J .. Nor-leucine, L-tyrosine and threonine.
M .. Methionine, aspartic acid and lysine.
P .. Phenylalanine, glutamic acid and serine.

Results.—The following Table shows in a tabular form the result of our experiments (column 2 gives us the *R_f* values of known amino acids while columns 4, 5, 7 and 8 give us the *R_f* values of the unknown bands):

* The combination of known amino acids kept at each spot was found out after a number of chromatograms were run with single drop of only one amino acid at each smaller circle. The sequence of these appearing on the standard size of the paper was determined and from these the combination was selected.

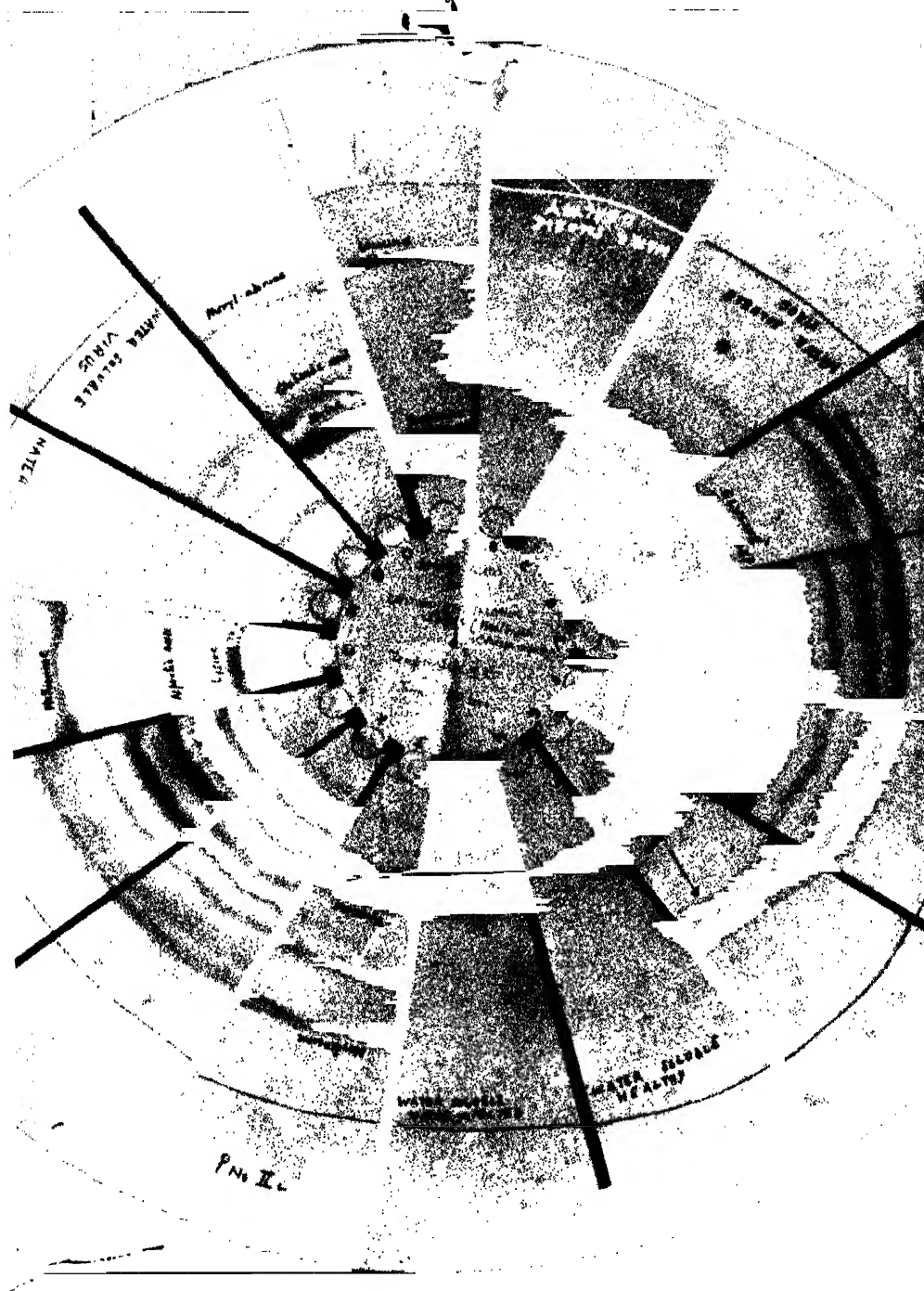


TABLE I

Known amino acid	Calculated <i>R_f</i> values	<i>R_f</i> values					
		Water-soluble			Hydrolyzed		
		Band No.	Healthy	Diseased	Band No.	Healthy	Diseased
Leucine and isoleucine.	0.78	X	X	X	I	0.78	0.78
Valine and methionine.	0.72	X	X	X	II	0.71	0.71
Phenylalanine ..	0.75	X	X	X	X	X	X
Tryptophan ..	0.69	X	X	X	X	X	X
D-L tyrosine and L-tyrosine.	0.62	I	0.62 (v. faint)	0.62	III	0.61	0.61
Proline ..		II	identified by its yellow colour and position (cf. Giri, 1952)			X	X
Alanine ..	0.53	X	X	X	IV	0.53	0.53
Glutamic acid and threonine.	0.46	III	0.46 (faint)	0.46	V	0.46	0.46
Glycine and aspartic acid.	0.40	IV	0.40 (faint)	0.40	VI	0.40	0.40
Serine ..	0.33	V	0.33 (deeper than others)	0.32	X	X	X
Arginine ..	0.25	X	X	X	VII	0.26	0.26
Histidine and lysine	0.22	VI	Absent	0.21 (Prominent)	VIII	0.23	0.23
Cystine ..	0.15	X	X	X	IX	0.15	0.15
Glutathione or cysteic acid.	..	VII	0.094 (v. faint)	0.094 (v. faint)	X	0.09	0.09

Confirmation.—All the amino acid bands were confirmed by adding known amino acids to the unknown solutions on separate chromatograms. The deepness in those rings as well as the constancy in the number of bands gave us the clue of all the amino acid bands identified.

In case of water-soluble extract the absence of glycine was found out by running phenol saturated with buffer pH 12 after the buffered paper (pH 12) was run on butanol: acetic: water solvent and dried.

The difference in our *R_f* values with those of others can be very well explained on the basis of the difference in the pH of the solvent, temperature, degree of saturation and the distance between starting point and the solvent boundary which in our

TABLE II

Known amino acid	Known <i>Rf</i> values	<i>Rf</i> Water-extract (Healthy)	<i>Rf</i> Water-extract (Diseased)	Colour of bands
Aspartic acid ..	0.57	0.56	0.56	Violet
Threonine ..	0.57	0.56	0.57	Violet
Glycine ..	0.48	X	X	Brown
Glutamic acid ..	0.44	0.44	0.45	Violet
Serine ..	0.43	0.43	0.43	Violet

case differs with those of other authors. But our results are consistent and the keeping of reference solutions has helped us in eliminating all these defects.

In some cases the bands do not appear as complete arcs. This being explained as due to spray defect which has been eliminated in newer work.

DISCUSSION

(a) *Method*.—The method described above has the benefit of Ganguli's improvement; that many solutions can be estimated and at the same time the radial diffusion is also maintained. In addition, the bands come deeper because of their restricted movement, even of those amino acids which are present in low concentrations. The choice of single wick as against multiple wick gave us better separation, for in the latter case the movement of solvent was too fast and the separation was not so marked as in our case.

(b) *Material*.—Because of the fact that both the diseased (stunted) and healthy plants were growing in the same nutritional conditions, it was concluded that they had no physiological yellowing. Many of the plants showed mosaic sort of pattern at the top while the lower leaves remained healthy. This gave us the hint that the causal organism was most probably not seed transmissible. The symptomatology indicates that the causal organism of the 'yellow-mosaic' is neither due to any physiological cause nor it is due to any fungus or bacteria, for the culture of the diseased leaves showed no indication of either of the two. Considering all these it is suspected that it is some virus (the study of which is also being tried with the methods available).

(c) *Interpretation of the result*.—From a study of Table I it is clear that the following free amino acids are present in normal healthy croton leaves:

Tyrosine, proline, glutamic acid and threonine, aspartic acid, serine, and glutathione or cysteic acid. (The last two are the only two substances whose *Rf* values are below that of cystine.)

These acids are also found in common with the 'yellow-mosaic' leaves which show common *Rf* values on our chromatograms. They, however, appear more prominently in the diseased leaves. But lysine and histidine which have common *Rf* values come out very prominently in the diseased leaves only. One would like to conclude from our results that in the normal metabolic flux of proteins, lysine or histidine do not take part in the intermediate steps. The formation of this new amino acid (having *Rf* value 0.22 corresponding to the lysine and histidine band) which is present in free state in the diseased leaves goes in agreement with the suggestion of Bawden (1954) who postulates synthesis of a new range of proteins due to virus infection. Whether, this amino acid, is a constituent of the changed metabolic flux of protein in the plant due to such an infection, or it is a step in the formation of virus protein is yet to be decided.

Further work is in progress.

SUMMARY

1. The work deals with the study of water-soluble amino acids and those obtained by hydrolyzing the protein content of the healthy and diseased leaves of *Croton sparsiflorus*.
2. Amino acid analysis made on circular papers which were divided into 16 sectors, by perforating 16 radial fins at equal distances, revealed that healthy leaves contained tyrosine, proline, glutamic acid and threonine, aspartic acid, serine and glutathione or cysteine acid.
3. 'Yellow-mosaic' leaves showed all those free acids found in common with the healthy ones but a band corresponding to lysine-histidine appears as new.
4. It is suggested that a new range of amino acid is formed due to the 'yellow-mosaic' condition of the leaves.

ACKNOWLEDGEMENT

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EXPLANATION OF PLATE

PLATE VI. Chromatogram showing amino-acid bands of healthy and 'diseased' leaf of *Croton sparsiflorus* along with known reference solutions.

- (1) Reference solutions: *A* .. Leucine, arginine and cystine.
D .. Isoleucine and glycine.
G .. D-L valine, D-L tyrosine, and histidine.
J .. Norleucine, L-tyrosine, and threonine.
M .. Methionine, aspartic acid and lysine.
P .. Phenylalanine, glutamic acid and serine.
- (2) Plant solutions: Water-soluble extract of healthy leaves at *B*, *H* and *N* and of diseased leaves at *C*, *I*, and *O* hydrolysate of healthy leaves at *E* and *K* and of diseased leaves at *F* and *L*.

ON SOME MALE FRUCTIFICATIONS REFERABLE TO *GLOSSOPTERIS* AND THE SYSTEMATIC POSITION OF THE GENUS

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(Communicated by S. M. Sircar, F.N.I.)

(Received January 15, 1955)

INTRODUCTION

The record of any undoubted fructification of *Glossopteris* has long been missing. The recent valuable contribution by Plumstead (1952) to the female fructifications of the genus has considerably added to our knowledge regarding its affinities. Since then, a few other *Glossopterid* female fructifications have also been described by the present author (Sen, 1954, unpub.). The morphological interpretation of these organs, however, remain controversial (Edwards, 1952; Walton, 1952; Plumstead, 1952; Sen, unpub.). It has been felt that the discovery of male organs of *Glossopteris* is now likely to settle its systematic position perhaps more conclusively. Fortunately such a possibility is now at hand.

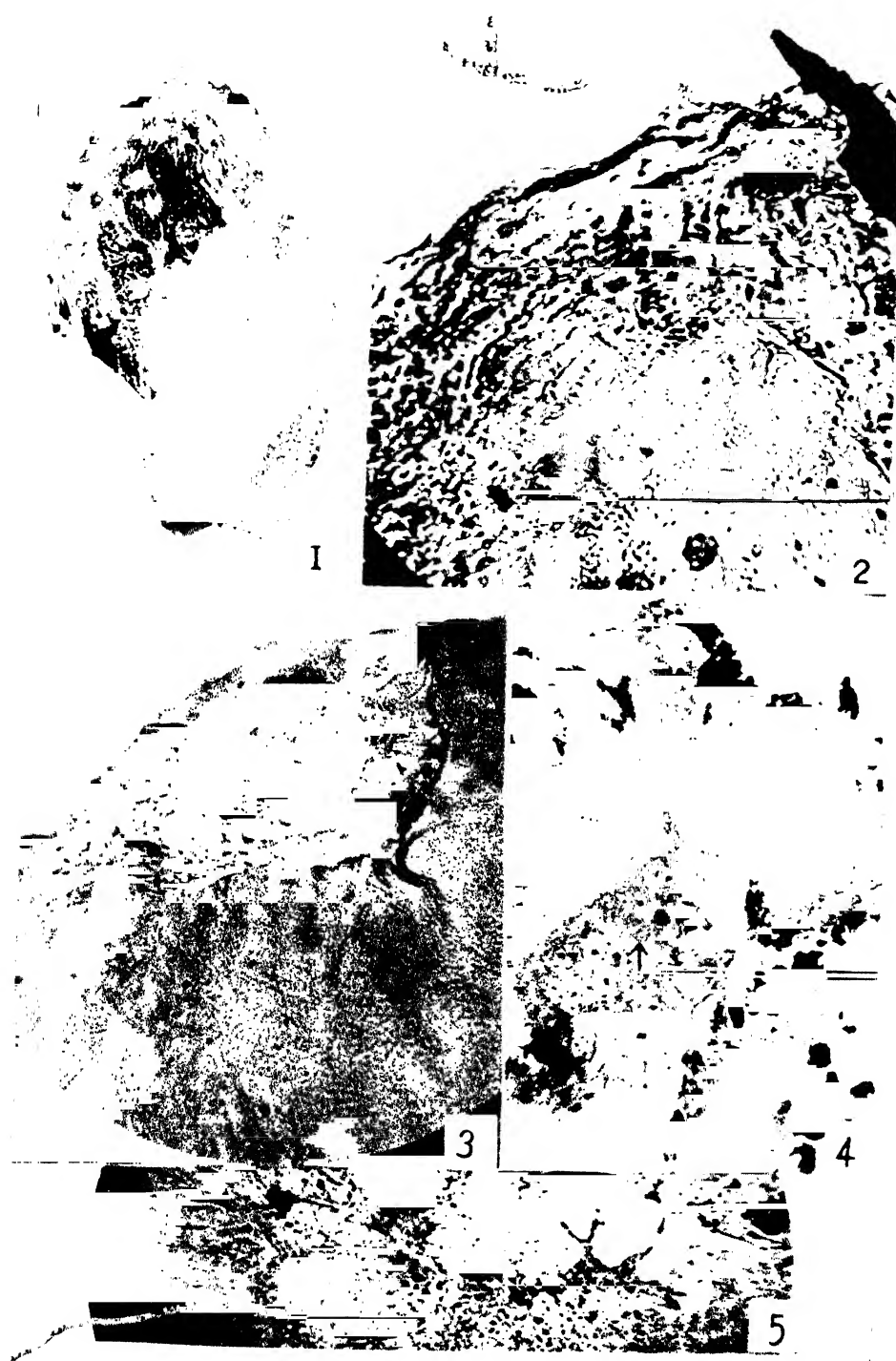
Recently a number of sacs containing typical *Pityosporites* type of pollen have been recovered from Indian Lower Gondwana coal. Since *Pityosporites* has been assumed to be the pollen of *Glossopteris* on grounds of their close association in the Lower Gondwana rocks in different parts of the southern hemisphere and particularly India (Virkki, 1945; Ghosh and Sen, 1948; Sen, 1948, 1953), it was thought that intense search for these sporangia-like structures (referable to *Glossopteris*) might be profitably pursued. Such a successful finding is also likely to add more definite clues to the long standing problem of the botanical affinities of *Glossopteris*.

A large number of analyses of Indian Lower Gondwana coals from the Ranigunj and Karharbari stages by the present author yielded a variety of sporangia-like structures, some of which have been found associated with or containing typical two-winged pollen of *Pityosporites* type (plates VII and VIII, figs. 1-5 and 6). When smeared under a cover glass, some of them liberated numerous *Pityosporites* type of pollen grains (plate VIII, fig. 11). These structures containing *Pityosporites* are as such probably related to *Glossopteris*. The pollen masses may also be teased out from pollen sacs (plate VIII, figs. 9-10), or they may occur freely in the maceration residues (plate VIII, fig. 8).

DESCRIPTION OF THE MICROSPORANGIA

The microsporangia are variable (probably belonging to different species) (plates VII and VIII, figs. 1-4 and 6), sac-like structures of peculiar form often looking like moss capsules. They occur freely and singly (possibly not otherwise obtainable in maceration), and taken as such they are unlike the sori of ferns. They appear to be sessile, somewhat elliptical, tapering at one extremity, the other end (possibly the attachment region) usually remaining relatively broad; often they look almost oval. They measure $800\ \mu$ - $1,500\ \mu \times 650\ \mu$ - $1,000\ \mu$.

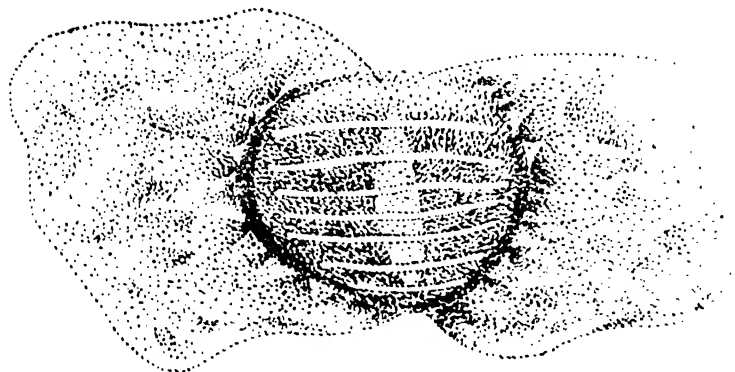
The sporangia are unilocular, dehiscing regularly more or less in transverse plane at the apical region (plates VII and VIII, figs. 1-4 and 6), but the subsequent tendency for dehiscence in a longitudinal direction is also sometimes evident (plates VII and VIII, figs. 4 and 6); all the sporangia appear exannulate. The cells of the





sporangia wall are usually long, regularly and/or irregularly rectangular to rhombic shaped, sometimes small and almost squarish to rectangular (plates VII and VIII, figs. 2-3 and 6-7), more often their outlines are destroyed. It has been found that small masses of *Pityosporites* are sometimes covered by torn pieces of tissues consisting of small squarish to rectangular cells (plate VIII, fig. 8) which are similar to the cells of a sporangium (plate VIII, fig. 6). The sporangial wall is usually very thin possibly due to the effects of maceration and as such hardly measures more than 1.5μ in thickness.

The pollen grains are bilateral with two reticulately marked characteristic bladders placed on opposite sides of the body which is surrounded by a thick rim (Text-fig. 1; plates VII and VIII, figs. 2, 5, 9 and 11-12). When the pollen grains occur in clusters or within the sacs they are probably not fully mature since their size is usually relatively smaller than those found free. The types of grain observed in masses and/or within the sacs range in total length (bladder tip to bladder tip) from 50μ to 100μ . The description of *Pityosporites* types of pollen, as above, is very common in palynological literature of the Palaeozoic (Schopf, Wilson and Benthall, 1944; Virkki, 1945; Ghosh and Sen, 1948; Sen, 1948; and others).



TEXT-FIG. 1. *Pityosporites* sp. A characteristic pollen inside the sac as shown in the Plate VII, fig. 2. $\times 862$.

PREVIOUS RECORDS OF MICROSPORANGIA REFERRED TO *GLOSSOPTERIS*

The microsporangia long known to be referable to *Glossopteris* are some empty and exannulate bodies reported by Arber as far back as in 1905. These so-called microsporangia occur in groups, and are probably borne on the lower concave surface of some transitional structures between scale and foliage leaves found in association with *Glossopteris browniana*. Arber compared his sporangia with those of a recent cycad, and that described by Zeiller as *Discopteris Ralli*. He also referred to the earlier suspicions regarding the presence of sori-like bodies found associated with *Glossopteris*. In this connection Feistmantel's (1881, 1882, 1886) reference to the sori or like bodies, which are variously arranged on the margin or surface of some species of *Glossopteris*, deserves special consideration. Unfortunately Feistmantel's descriptions are very brief and his illustrations are not in sufficient detail so that they can be profitably compared with the present findings.

Subsequently in 1932, Du Toit described *Eretmonia natalensis* which might be the organ bearing the types of microsporangia already referred to by Arber (1905) and also described by Seward (1907). Du Toit interpreted that his find was most probably the male reproductive organ of a species of *Glossopteris*.

Seward and Sahni's (1920) reinterpretation of *Ottokaria bengalensis* suggested that this specimen was probably the ovulate organ belonging to a pteridosperm. But Plumstead (1952) does not altogether rule out the possibility of the species being the staminate reproductive organ of a *Glossopteris*, which view is, however not at all suggestive because *Ottokaria bengalensis* appears more as 'a cupular investment of a seed'.

In 1947 Teixeira illustrated a leaf of *Glossopteris indica* with two rows of projections, 'regularly arranged, alternate, recalling the sori of certain ferns'. A fragment of a leaf of *G. angustifolia* showing similar structures had earlier been described by Zeiller (1896). Apparently these structures cannot be closely compared to the microsporangia described in this paper.

DISCUSSION

Whenever a Lower Gondwana coal or shale (bearing *Glossopteris*) has been analysed, *Pityosporites* usually appears to be the constant and often the dominating feature of the microfloral yield, like the species of *Glossopteris* in the coal-bearing Permian and U. Carboniferous flora of the southern hemisphere (Virkki, 1945; Ghosh and Sen, 1948; Sen, 1948, 1953). The close association of *Pityosporites* with *Glossopteris* has more clearly been manifested in the eastern part of the Raniganj coalfield (Ghosh and Sen, 1948). In this field the number and types of *Pityosporites* gradually decline in successively higher beds, i.e., in seams nearer Panchet where *Glossopteris* is also on the decline. The Raniganj coal measure is characterized by about 10 species of *Glossopteris* whereas in Panchet there are only 4 species. Hence there are fewer number and types of *Pityosporites* in the upper portion of the coal measure where *Glossopteris* is fast vanishing. Virkki (1945) also obtained a few *Pityosporites* adhering to small pieces of cuticle of *Glossopteris browniana* from a macerated piece of shale from the Permo-Carboniferous rocks of Newcastle, New South Wales. It may not be very wise to underrate such a close association of *Pityosporites* and *Glossopteris* existing over a great length of time and spread over an extensive area. If *Pityosporites* is finally accepted as pollen of *Glossopteris*, it is logical to conclude that the structures bearing *Pityosporites* are microsporangia of *Glossopteris*.

Schopf *et al.* (1944) hold that there can be little question that the grains of the *Pityosporites* type are referable to coniferae. At the same time they recognized the diversities of forms among the *Pityosporites* which according to them possibly contain elements of separate families. A step to segregate such an artificial and heterogeneous genus into more natural units has recently been taken up (Kosanke, 1950).

It is definite that all spores so long recognized as *Pityosporites* do not belong to the same close circle of affinity, and as such most of the species of *Pityosporites* reported from the *Glossopteris* bearing Lower Gondwana rocks of the southern hemisphere are at least different from those occurring elsewhere. Moreover, the coniferous affinity of *Pityosporites* is solely based on theoretical assumption. Under the circumstances the sporangia containing typical Gondwana *Pityosporites* appear to be referable to *Glossopteris* despite the former's hypothetical relationship to the conifers. The short account on the botanical affinities of the *Glossopteris* that follows is based on this assumption.

Glossopteris fronds are often found in association with small seeds (Walton, 1940) and *Vertebraria* stem possessing secondary wood with multiseriate bordered pitted tracheids and parenchymatous medullary rays of gymnospermous type (Walton and Wilson, 1932). However, it is now known that most probably *Glossopteris* and *Vertebraria* are not parts of the same plant (Thomas, 1952a). But on the basis of extraordinary types of ovulate organs recently found attached to some species of *Glossopteris* (Plumstead, 1952) 'it may be regarded as a pteridosperm,

if that term is used in a very broad sense; but it differs from all the known plants of this type in both vegetative and reproductive characters' (Thomas, 1952b). Professor Harris (1952) has also doubted the propriety of including such plants under pteridosperms. He has further felt that Plumstead's (1952) interpretation of the ovulate organ of *Lanceolatus* type would suggest this plant to be an angiosperm.

The simple unilocular sporangia containing *Pityosporites*, as described in this paper, appear to be akin to those of the primitive gymnosperms. The pollen of these sporangia, i.e., *Pityosporites*, superficially resembles that of abietineae as suggested by Schopf *et al.* (1944), but the chances of *Glossopteris* being leaves of a conifer appears to be a remote possibility. Moreover, the pollen sacs, as found by the author, are not at all abietinean.

It is apparent that an assemblage of detached organs consisting of *Glossopteris* fronds, simple microsporangia described in this note, and cupular organs protecting the seeds, which are found attached to *Glossopteris* by Plumstead (1952), point more to a pteridospermous nature than to any other group of plant. Some suggestions and reinterpretation of the morphology of ovulate organs have since been made to settle the systematic position of the genus (Edwards, 1952; Walton, 1952; Sen, unpub.).

SUMMARY

The pollen sacs of *Pityosporites* type of pollen have been described from Indian Lower Gondwana coal. On grounds of very close association throughout the Lower Gondwanas *Pityosporites* has long been thought to be the pollen of *Glossopteris* and therefore, the sac-like structures containing this type of pollen may belong to *Glossopteris*. There has so far been no indubitable record of the male organs of this plant.

This new report has added some interesting generalizations to the existing assumption as to the affinities of *Glossopteris*.

ACKNOWLEDGEMENTS

I am grateful to Professor Olof H. Selling, Naturhistoriska Riksmusem, Stockholm, for kindly going through the MS., and suggesting valuable improvements. Dr. K. Jacob of the Geological Survey of India, Calcutta, has also kindly read the MS.

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EXPLANATION OF PLATES VII AND VIII

Microsporangia containing or associated with *Pityosporites* sp.

- FIG. 1. A sporangium containing a few *Pityosporites* type of pollen (shown by arrow heads). Note the regular nature of apical dehiscence. $\times 70$.
- „ 2. The enlarged view of the apical portion of the fig. 1. Note the typical *Pityosporites* type of pollen inside the sporangium and the nature of its cell wall. $\times 280$.
- „ 3. The enlarged view of the apical portion of a sporangium. Note the juxtaposed *Pityosporites* type of pollen grains near the dehiscent mouth (forming a black mass) of the sporangium, and the disorganized nature of its wall cells. $\times 280$.
- „ 4. A sporangium containing some *Pityosporites* type of pollen (shown by arrow heads). $\times 50$. Note the nature of longitudinal dehiscence of the sporangium which appears to be of subsequent development leading to collapse after apical dehiscence.
- „ 5. The enlarged view of a portion of the inner surface of the fig. 4 showing *Pityosporites* type of pollen and faintly visible cell wall outlines. $\times 250$.
- „ 6. A sporangium with characteristic apical dehiscence and a subsequent tendency for dehiscing in longitudinal direction. $\times 52$. Note the shapes of the cells at the base of the sporangium and compare with those in the figure 6.
- „ 7. The enlarged view of the basal portion of the fig. 6 showing the nature of wall cells. $\times 110$. Compare fig. 8.
- „ 8. An irregularly torn compact mass of *Pityosporites* type of pollen under a cellular covering. $\times 65$. Since it is a sub-spherical structure it has not been possible to photograph both the covering and the distinguishable pollen at the edges of the mass. Note the shapes of the cells of this covering tissue and compare with those at the base of the sporangium in the fig. 6 and also those in the fig. 7.
- „ 9. A typical *Pityosporites* type of pollen somewhat isolated from the tissue matrix. $\times 215$.
- „ 10. A compact mass of *Pityosporites* type of pollen teased out of a sac-like body. $\times 70$.
- „ 11. A smeared semi-carbonized sporangium showing scattered pollen of *Pityosporites* type. $\times 60$.
- „ 12. The enlarged view of a portion of the fig. 11 showing typical *Pityosporites* type of pollen grains. $\times 165$.

EMBRYOLOGICAL STUDIES IN MALVACEAE—II

FERTILIZATION AND SEED DEVELOPMENT

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INTRODUCTION

In a previous article the writer (1954a) described the development of the anther, ovule and the gametophytes in fifteen species belonging to Malvaceae. In this paper fertilization, development of endosperm, embryo and seed are described in the following eight species: *Sida cordifolia* L., *S. veronicaefolia* L. (= *S. humilis* Willd.), *Abutilon indicum* G. Don., *Pavonia zeylanica* L., *Malachra capitata* L., *Hibiscus solandra* L. Herit., *H. hirtus* L. and *H. micranthus* L.

MATERIAL AND METHODS

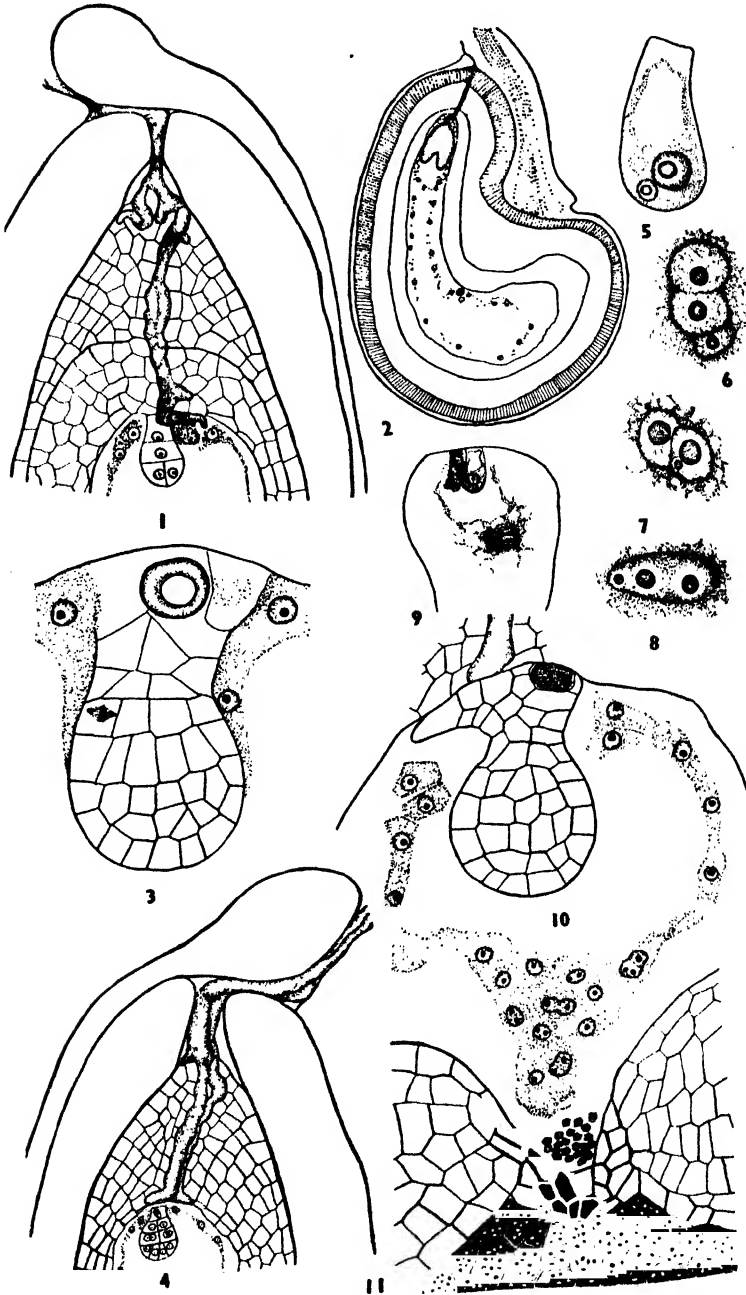
The material of *Malachra capitata* was obtained from Kakinada and the rest were collected locally. Sections were cut according to usual methods of micro-technique and stained with Delafield's and Heidenhain's haematoxylin.

Fertilization and early stages of embryogeny have been found to proceed at a rapid rate in all investigated Malvaceae. Therefore for a close study of these aspects, open flowers of *Hibiscus solandra* were labelled and the developing fruits were fixed at hourly intervals.

In all species studied, the stylar branches terminate in globose stigmas with either short (*Sida*) or elongated and tapering (*Hibiscus*) one-celled hairs. The styles which are solid (except in *Abutilon*) are provided with strands of transmitting tissue consisting of elongated, thin walled, richly protoplasmic cells full of starch grains. Stomata are found in the epidermis of the style and druses in the inner cells.

The ovules in *Pavonia zeylanica* and *Malachra capitata* are basal and erect, with the micropyle pressing against or standing very close to the base of the loculus or placenta, and the pollen tube enters the micropyle directly from the tissue of the placenta along which it traverses. In *Abutilon indicum*, however, several multicellular richly protoplasmic hairs arise from the base of the style and project into the cup-shaped cavity of the ovary below and function as an obturator. In *Hibiscus solandra*, similarly, a number of hairs arise from the placenta and bridge the gap between the placenta and micropyle, thus facilitating the entry of the pollen tubes into the ovules. Stenar (1925) also recorded the occurrence of such hairs in *Modiola caroliniana* which become especially prominent in fertilizable ovaries, though he did not ascribe any function to them. Such a hairy obturator is also seen in other Malvales like *Buettneria herbacea* of Sterculiaceae (C. V. Rao, 1954b) and *Triumfetta rhomboidea* of Tiliaceae (C. V. Rao and K. V. S. Rao, 1952).

The pollen grains are large and their cytoplasm is packed with reserve food in the shape of fat, aleurone grains and starch. As the pollen grains germinate these food materials pass into the pollen tubes, giving them a richly granular appearance and obscuring the gametic nuclei. The pollen grains are lodged in



FIGS. 1-11. Fertilization and endosperm development in Malvaceae.

FIG. 1. Micropylar part of the ovule of *Hibiscus micranthus* showing branching pollen tube. $\times 175$.

„ 2. L.S. of developing seed of *Abutilon indicum* showing embryo with cotyledon primordia, endosperm which has become cellular around the embryo and persistent pollen tube. $\times 30$.

large numbers on the stigma and germinate within a few minutes of pollination. Self-pollination occurs in *Hibiscus solandra*. Pollen tubes were already formed in flowers plucked just after opening. Flower buds enclosed in cellophane paper bags

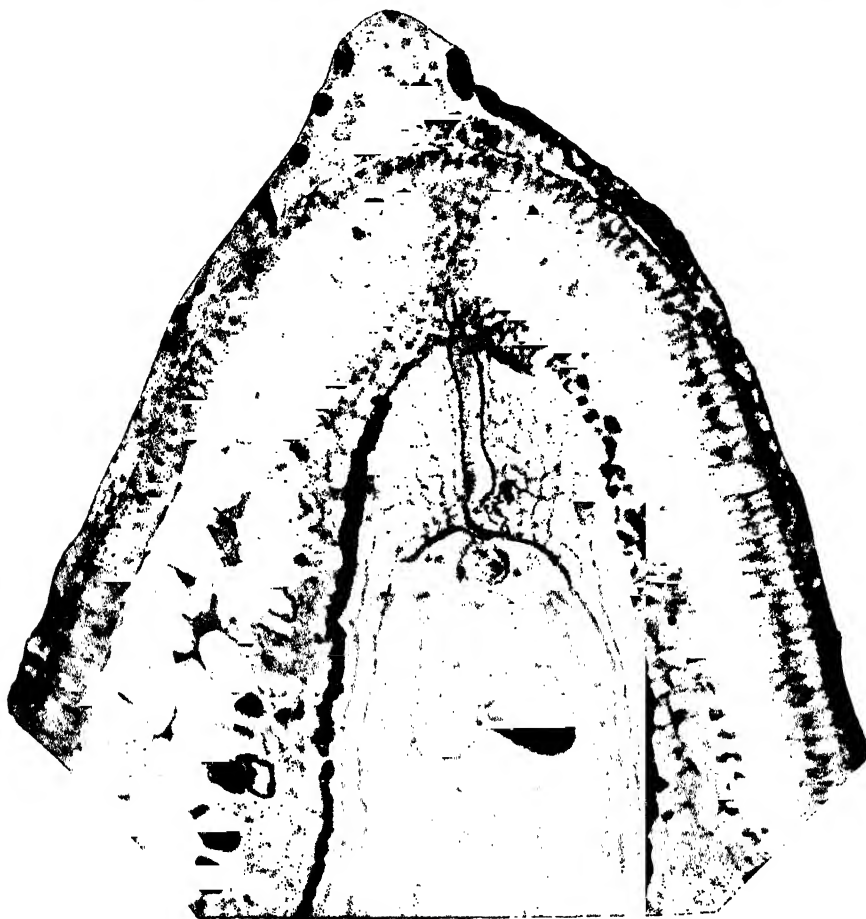


FIG. 12.

FIG. 12. Microphotograph of L.S. of upper part of the seed of *Hibiscus solandra* showing embryo, persistent pollen tube and tannin and starch bearing cells of the integuments. $\times 165$.

FIG. 3. Micropylar part of the embryo sac of *Hibiscus hirtus* showing globular embryo and the cut end of pollen tube which looks like a tracheid. $\times 370$.

„ 4. Micropylar part of the ovule of *Hibiscus hirtus* showing penetration of two pollen tubes. $\times 175$.

„ 5. Fertilization of egg in *Abutilon indicum*. $\times 370$.

FIGS. 6-8. Formation of the primary endosperm nucleus in *Pavonia zeylanica*. $\times 780$.

FIG. 9. Upper part of the embryo sac of *Hibiscus micranthus* showing fertilized egg and first division of the primary endosperm nucleus. $\times 175$.

„ 10. Micropylar part of the embryo sac of *Hibiscus micranthus* showing embryo with boot-shaped suspensor, endosperm which has become cellular, persistent pollen tube and remnant of a synergid. $\times 250$.

„ 11. Antipodal end of embryo sac of *H. micranthus* showing super-numerary persistent antipodals, endosperm (note nuclear fusions), and food bearing cells of the chalaza. $\times 250$.

fruited in due course. As Stenar (1925) and Lang (1937) have already noticed, the emergence of pollen tubes is polysiphonous. Stenar (1925) suggested that the accessory pollen tubes might be serviceable in keeping the large pollen grains anchored to the stigma by getting entangled with the stigmatic hairs.

The pollen tubes in *Hibiscus solandra* reach the base of the style in about four hours of pollination. After reaching the tip of the nucellus, they give off a few short branches which end blindly, while the main tube carrying the gametic nuclei progresses towards the embryo sac (Fig. 1). The pollen tubes in Malvaceae are especially interesting. They are 15–20 μ wide and persist till a very late stage in the development of the seed (Figs. 2, 12). They do not collapse after discharging their contents but remain intact. They are lined with protoplasm-like material and their wall is pretty thick; its avidity for safranin might indicate that it is lignified. In one section (Fig. 3), its tip had been cut transversely by chance and it appeared very much like a tracheid. The structure and behaviour of the pollen tube suggest that it may serve a nutritive function by acting as a channel for the transport of food materials from the starch bearing cells of the integuments to the growing embryo. Longo (1903) in *Cucurbita* and Foster (1943) in *Carica papaya* also have credited the pollen tube with a similar function. In several cases, two pollen tubes were seen to enter an ovule (Fig. 4) as Iyengar (1938) also found in *Gossypium*, though polyspermy was not noticed.

The male gametic nuclei figured by Stenar (1925) in *Malva rotundifolia* and *Lavatera thuringiaca* (his text-figs. 52–55) do not show nucleoli; but in the present studies these nuclei were found to show distinct nucleoli at the time of fertilization. Like the nucleolus of the egg (and pollen mother cells), these nucleoli also show densely chromatic peripheral and a vacuole-like central region (Fig. 5).

Fertilization occurs after the formation of the primary endosperm nucleus or after its division. At first, the egg as well as the male gamete contain nuclei which are in the resting condition but just before syngamy a 'spireme' can be made out in both the nuclei. The nucleus of the fertilized egg shows dense chromatin and usually two but occasionally three nucleoli. Stages in syngamy are met with in material of *Hibiscus solandra* fixed 10–12 hours after pollination.

ENDOSPERM

The polar nuclei in all investigated species stand pressed together but do not fuse before fertilization. The male nucleus fuses first with one polar nucleus and then fusion occurs with the second polar to form the triploid 3-nucleolate primary endosperm nucleus (Figs. 6–8), as was also noticed in members of Sterculiaceae (C. V. Rao, 1954b) and Tiliaceae (C. V. Rao and K. V. S. Rao, 1952; Banerji, 1933). The primary endosperm nucleus divides within 12–15 hours of its formation in *Hibiscus solandra* and 4–8 endosperm nuclei are already formed when the fertilized egg undergoes the first division. The spindle during the first division of the primary endosperm nucleus in *Hibiscus micranthus* is oriented transverse to the embryo sac (Fig. 9).

The developing endosperm shows accumulations around the embryo and at the antipodal end of the sac, while it is relatively thin at the sides. Cell wall formation commences in the endosperm from the micropylar end when the embryo has reached the size of a fairly large globular mass or is already showing the cotyledonary primordia, and proceeds towards the antipodal end (Fig. 10). The nucellus about this time is almost crushed out at the sides of the embryo sac. In the micropylar region, however, it persists for a longer time, with the pollen tube intact, though the cells are devoid of cytoplasm. The endosperm in the antipodal part extends into the socket of thick walled cells described in the previous paper and seems to draw directly upon the reserve food contained in the cells of the chalaza (Fig. 11). In *Hibiscus hirtus* and *H. micranthus*, the antipodals do not degenerate after

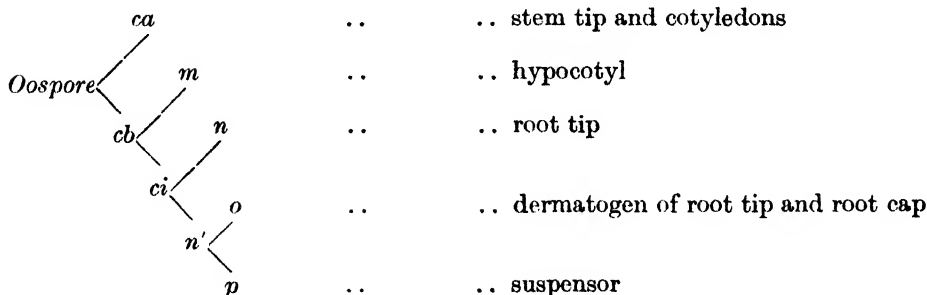
fertilization. On the other hand, they divide to form about 25–50 cells (Fig. 11). These antipodal cells are much smaller than the surrounding nucellar cells and stain deeper. As they stand between the endosperm and the food bearing cells of the chalaza, they seem to help in the transport of food into the sac, just as the pollen tube facilitates food transport at the micropylar end. Such antipodals are seen in highly evolved families like Rubiaceae, Compositae and Gramineae. In the antipodal part of the endosperm nuclear fusions sometimes occur which result in large sized polyploid nuclei (Fig. 11), as are also seen in some members of Sterculiaceae like *Abroma augusta* and *Pentapetes phoenicea* (C. V. Rao, 1954b).

The endosperm during cell formation first gets cut up into uninucleate protoplasts by a process of furrowing or indentation (Fig. 10) as Gore (1932) described in cotton. Later the cell walls are secreted. The embryo sac at this time is very large and the cytoplasm relatively scanty. It continues to enlarge rapidly even afterwards so that the nucellus of the ovule is completely eaten up. No perisperm is found in the seed. In the mature seeds of *Hibiscus micranthus* (Fig. 125) almost the whole of the endosperm is consumed; in *Sida cordifolia* (Fig. 28), *S. veronicaefolia* (Fig. 46), *Pavonia zeylanica* (Fig. 61) and *Abutilon indicum* (Fig. 71), however, a little of it could be seen in the fully formed seed.

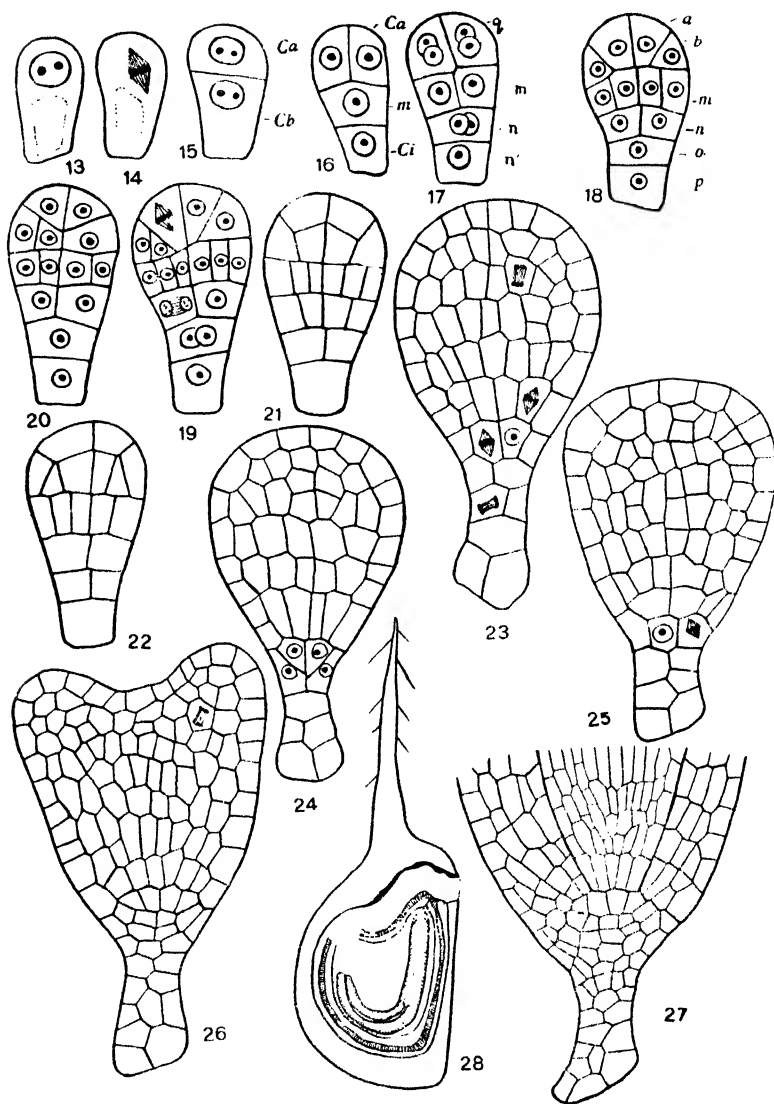
EMBRYOGENY

The development of the embryo occurs in a uniform manner in all species studied, except for slight variations. It will be described in *Sida* species and any minor variations will be pointed out in the remaining species. The development keys out to the *Urtica* variation of the Asterad Type.

Sida cordifolia L. and *S. veronicaefolia* L.—The fertilized egg (Fig. 13) divides transversely (Fig. 14) and gives rise to the terminal cell *ca* and the basal cell *cb* (Figs. 15, 29). The two cells next undergo division simultaneously, *ca* dividing longitudinally and *cb* transversely so that a 4-celled T-shaped embryo is formed (Figs. 16, 30). The two cells formed by *cb* are designated *m* and *ci*. The two cells of the terminal tier now divide in a vertical manner in a plane perpendicular to that of the first division forming quadrants termed *q* (Figs. 17, 31). *M* divides vertically and *ci* transversely forming two cells designated *n* and *n'* (Figs. 17, 31). The quadrants of the tier *q* divide in an oblique manner giving rise to four centrally placed cells termed *a* and four peripherally placed ones called *b* (Figs. 18, 32). The walls of these cells may touch the horizontal (Fig. 18) or vertical wall of this tier (Fig. 20). Vertical divisions occur in the cells of *m* which result in formation of circumaxially arranged quadrants. *N* also divides vertically into two cells (Figs. 18, 33) and *n'* by a transverse division gives rise to two cells *o* and *p* (Fig. 18). The embryo at this stage is 16-celled and 5-tiered as in Sterculiaceae and the destination of the tiers is also similar as shown in the following schematic representation:



After this stage, the quadrants of *m* undergo periclinal divisions to demarcate the dermatogen initials to the outside and the common periblem-plerome initials

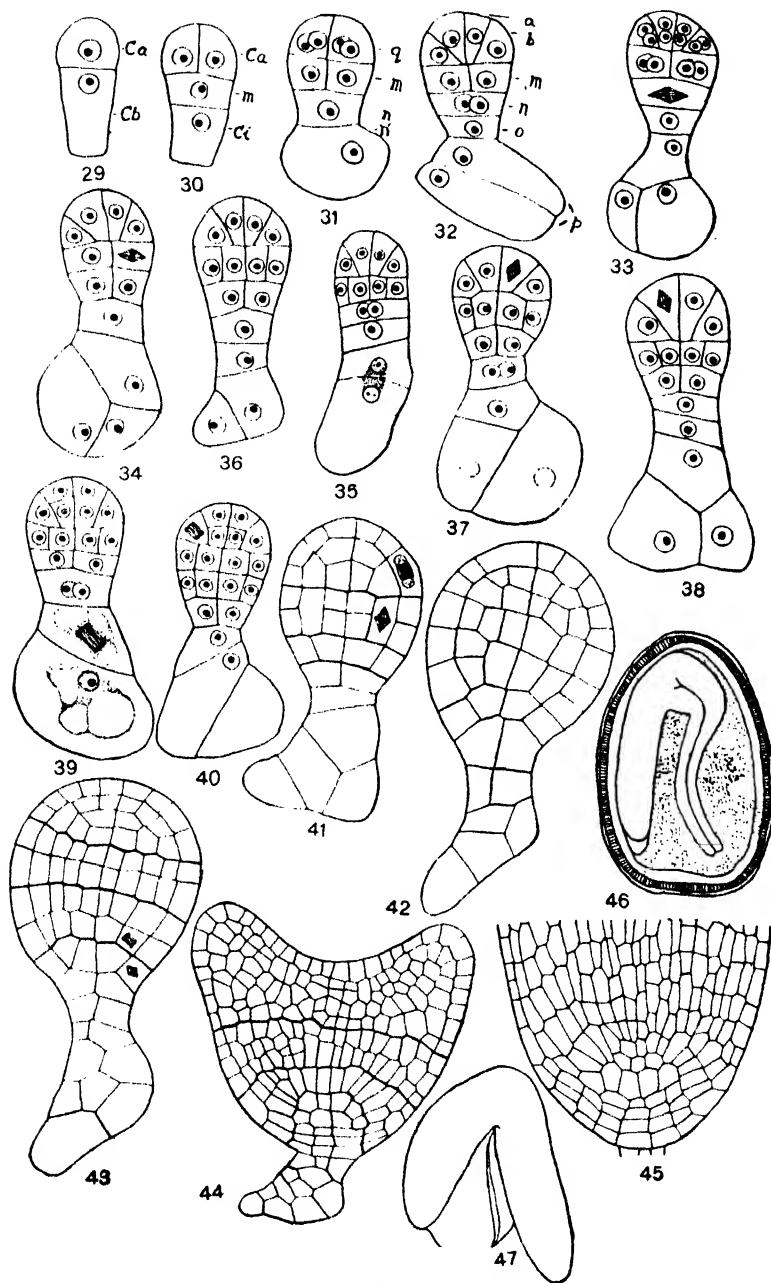
FIGS. 13-28. *Sida cordifolia*.

FIGS. 13-26. Various stages in the development of the embryo. $\times 330$.

FIG. 27. Lower part of the embryo showing suspensor, root tip and root cap. $\times 215$.

„ 28. L.S. of fruitlet. $\times 10$.

to the inside (Figs. 18, 19, 34, 35). The demarcation of dermatogen in the terminal tier occurs first in the cells of *a* and then in the group of cells *b* (Figs. 19, 37-40), though sometimes the reverse happens (Fig. 20). Divisions in *n* occur in the same manner as in *m* and result in the demarcation of the three histogenic layers (Figs. 20, 21, 39, 40). *O* functions as the hypophyseal cell; at first it undergoes two vertical divisions which result in the formation of a plate of four cells (Figs. 21, 22, 40). These cells next undergo oblique divisions (as occur in the terminal tier), forming a group of four cells to the inside, and four to the outside which function,

FIGS. 29-47. *Sida veronicaefolia*.FIGS. 29-43. Various stages in embryo development. $\times 355$.FIG. 44. Embryo with cotyledon primordia. $\times 240$.,, 45. Part of the root tip of mature embryo. $\times 240$.,, 46. L.S. mature seed. $\times 10$.,, 47. Entire embryo. $\times 10$.

as the root cap initials (Figs. 23, 24). In *Sida cordifolia*, the group of cells formed to the inside undergo another division giving rise to a second layer of root cap initials to the outside and the dermatogen initials of the root tip to the inside. The two layers of root cap initials undergo periclinal and anticlinal divisions so that the root cap of the mature embryo becomes several layered (Figs. 25–27, 44, 45).

P builds up the suspensor. In *Sida veronicaefolia*, it is vesicular and richly protoplasmic. It also undergoes the first division earlier than in other species, even before *o* divides (Fig. 32). The first formed cells are large and glandular and equal in volume the whole of the embryonal mass (Figs. 39, 40). By successive divisions they become smaller and smaller (Figs. 41–43). They are not vesicular in *Sida cordifolia* and resemble those in other members of the family like *Abutilon indicum* and *Pavonia zeylanica*. In all cases, the suspensor is large and somewhat boot-shaped (Figs. 27, 42–44) in the fully formed embryo. It differs in this respect from that of the remaining families of Malvales. It seems to be a specialized organ exposing a large area for absorption of food materials which the rapidly growing embryo needs. Schnarf's (1931) remark that 'in general Malvaceae seem to possess a strikingly short suspensor' seems to be based on inadequate data and is no longer quite correct.

The mature seed shows a large embryo with foliaceous folded cotyledons (Figs. 28, 46, 47). The cells of the embryo are packed with reserve food materials in the form of starch and protein.

Pavonia zeylanica L.—The segmentation of the oospore and the derivation of the embryonic organs follow the same course as in *Sida*. The hypophysial cell divides relatively late in development of the embryo. The suspensor in this species remains 1-celled for a relatively longer time. It undergoes the first division after *n* has formed the quadrants. The suspensor cells remain small from the beginning.

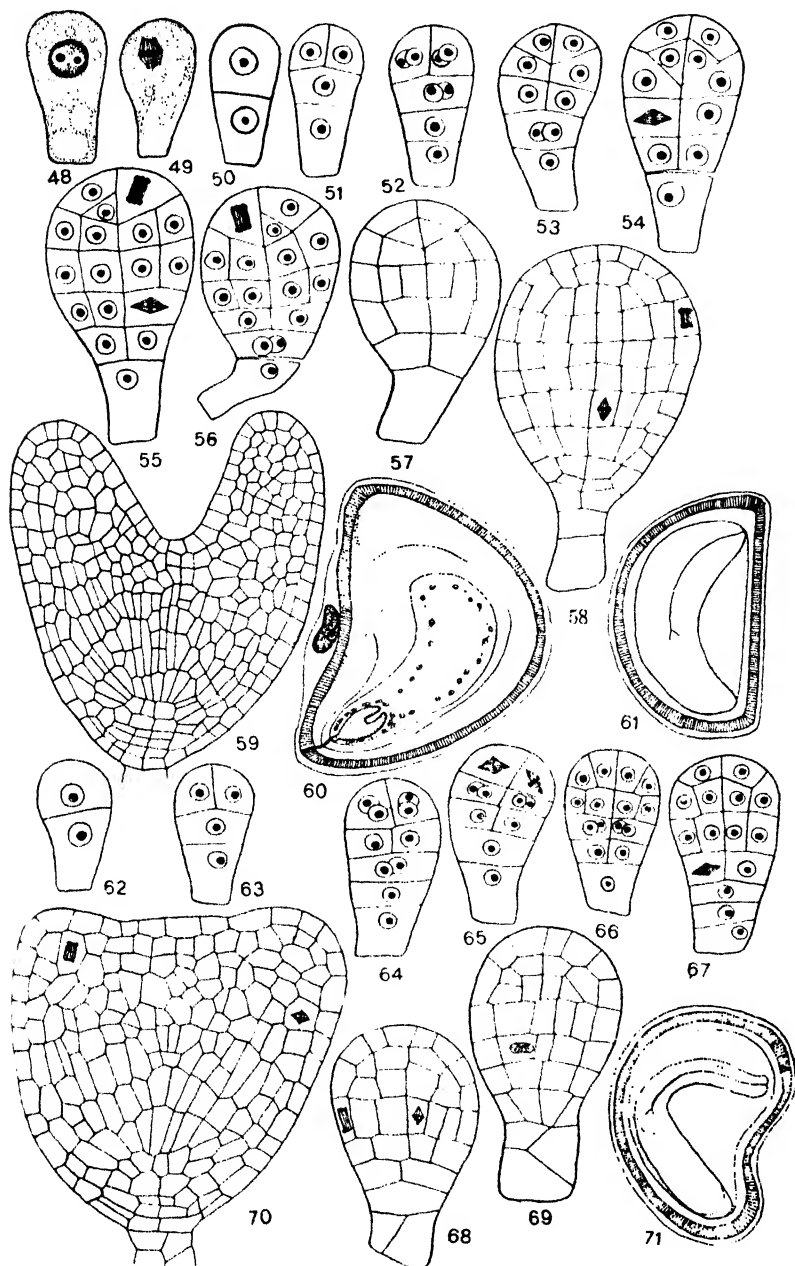
Abutilon indicum G. Don.—Development of the embryo follows closely that in *Sida* and *Pavonia*. The suspensor cells are not vesicular. The root cap initiating layers are variable being one or two as Souèges (1922) also found in *Malva rotundifolia*.

Malachra capitata L.—This species resembles *Pavonia zeylanica* in the organization of the suspensor. The rest of the embryo development is the same as in the other species studied.

Hibiscus solandra L. Herit., *H. hirtus* L., and *H. micranthus* L.—The young embryos in *H. solandra* appear more elongated than those of the other two species of *Hibiscus*. In *H. hirtus* and *H. micranthus* the suspensor cell of the young embryo is somewhat vesicular (Figs. 101, 113). In *H. solandra* occasionally a slight variation is seen in the segmentation of the cells *a* of the tier *q*. Ordinarily these cells divide transversely cutting off the dermatogen initials to the outside but sometimes one of these cells divides in an inclined manner so as to form a cell that looks like an epiphyseal cell (Fig. 89). In subsequent divisions, however, the derivatives of this cell become indistinguishable from the rest. Such deviations have been noticed by Souèges (1922) also in *Malva rotundifolia*.

One abnormal embryo was found in *Hibiscus solandra* (Fig. 88). The cells of the tier *q* in this case, appear normal but those of the remaining tiers instead of showing dense cytoplasm characteristic of embryonal cells, showed vacuolated cytoplasm and hypertrophied nuclei with large nucleoli. The cells of the sub-apical tier were much elongated; while one cell had divided longitudinally and demarcated the dermatogen initial, another (in sectional view) had divided in a transverse manner. A similar transverse division had also occurred in one of the cells derived from *n*.

The suspensor in *H. hirtus* (Fig. 106) and *H. micranthus* (Fig. 118) becomes large and boot-shaped earlier than in *H. solandra*. In *H. hirtus* *o* divides relatively later than in other species of the genus in which respect it resembles *Pavonia zeylanica*. The number of root cap initiating layers is variable but more commonly



FIGS. 48-71.

- FIGS. 48-61. *Pavonia zeylanica*. FIGS. 48-58. Stages in embryo development, $\times 320$. FIG. 59. Embryo with cotyledon primordia, $\times 215$. FIG. 60. L.S. of a developing seed showing embryo with cotyledon primordia, endosperm aggregation around the embryo which has become cellular and a persistent pollen tube, $\times 30$. FIG. 61. L.S. of mature seed, $\times 6$.
- 62-71. *Abutilon indicum*. FIGS. 62-69. Stages in embryogeny, $\times 320$. FIG. 70. Embryo with cotyledon primordia, $\times 215$. FIG. 71. L.S. mature seed, $\times 6$.

it is two. The embryo in the fully formed seed is large and nearly fills the seed cavity, the little endosperm left, being confined to the folds of the cotyledons.

SEED COATS

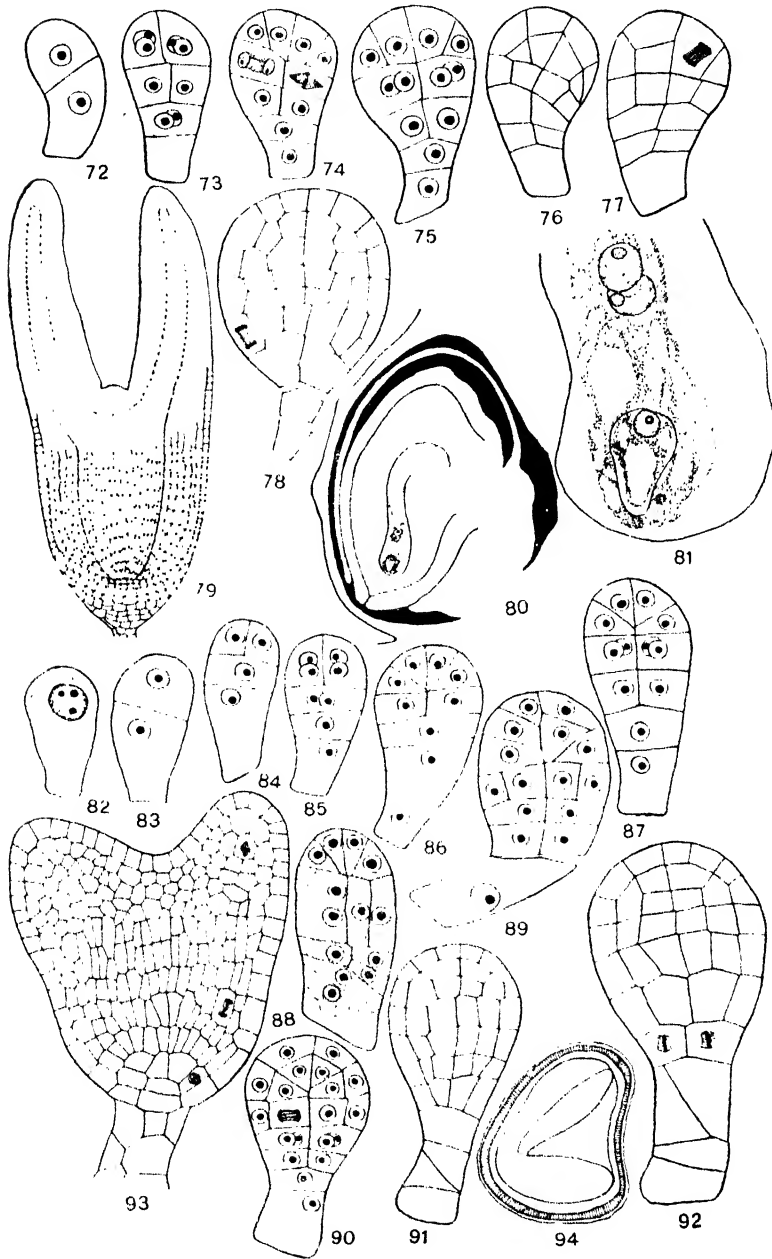
The writer's observations on the development and structure of the seed coats in *Sida cordifolia*, *Pavonia zeylanica*, *Malachra capitata*, *Abutilon indicum* and *Hibiscus* species are in close agreement with those of Reeves (1936).

The outer and inner integuments of the fertilizable ovule are usually 2-3 and 5-6 cells thick respectively, with an air space in between. After fertilization, the inner integument becomes 8-10 layered while the outer remains of the same thickness and forms a membranous testa, and the air space disappears. In some members of the family, like *Hibiscus micranthus* and *H. hirtus*, the testa develops hairs while in others including *H. solandra*, it remains smooth. In all species at first the outer epidermis of the outer integument in the fertilizable ovule consists of uniform isodiametric cells. In species which develop epidermal hairs, when the embryo has become 8-16 celled, some of the epidermal cells become conspicuous by their larger size, denser cytoplasm and more prominent nuclei. These cells occur in pairs; each cell then divides asymmetrically and forms a larger and a smaller cell. So the cells lie in linear rows of four before the development of hairs (Fig. 119). The nucleus of the elongating cell may divide once mitotically (Fig. 120). The lumen of the mature hair gets filled with deep staining matter (Fig. 121). In the remaining species with smooth testa, the cells of the epidermis of the outer integument are more tangentially flattened and devoid of cytoplasm and filled with tannin. In *Hibiscus solandra* and *Pavonia zeylanica*, the cells do not develop into hairs but become papillate. In the latter species, the tannin filled, deep staining cells are so arranged among the colourless cells as to give a pattern of rectangles to the surface view of the testa.

The thickness of the tegmen varies in different parts of the same seed. In the region of the micropyle it is thinner, while on the side of the funicle and chalaza it is thicker (Fig. 60). The cells of its outer epidermis are at first isodiametric and richly protoplasmic. After the embryo becomes globular, the cells begin to elongate radially and develop into the palisade layer (Figs. 122, 123). Ultimately they become very thick walled and show a prominent light line. The lignified inner half of the cell wall stains with phloroglucinol while the outer half which consists of cellulose does not. The lumen of the cell with degenerate remnants of the protoplast is confined to the outer half of the cell (Fig. 124). The cell wall is striated. One or two layers of large tannin and starch bearing cells immediately below the palisade layer constitute the 'pigment layer'. These cells are more numerous in the region of the micropyle and on the side of the funicle. The cells between the pigment layer and the inner epidermis of the inner integument are large, thin walled and parenchymatous (Fig. 12) and get crushed in the developing seed. The inner epidermis of the inner integument termed the 'fringe tissue' consists of tangentially flattened cells. Their cell walls stain deeply with safranin and appear to be ligno-suberized, as Reeves (1936) also noted. Their lumens contain tannin which stains very deeply. The tegmen of the mature seed consists of the palisade layer, which accounts for more than half the thickness of the seed coats, the pigment layer and the fringe tissue with the remnants of the crushed cells in between. In general there is a close similarity in the development and structure of the seed coats in all the families of Malvales.

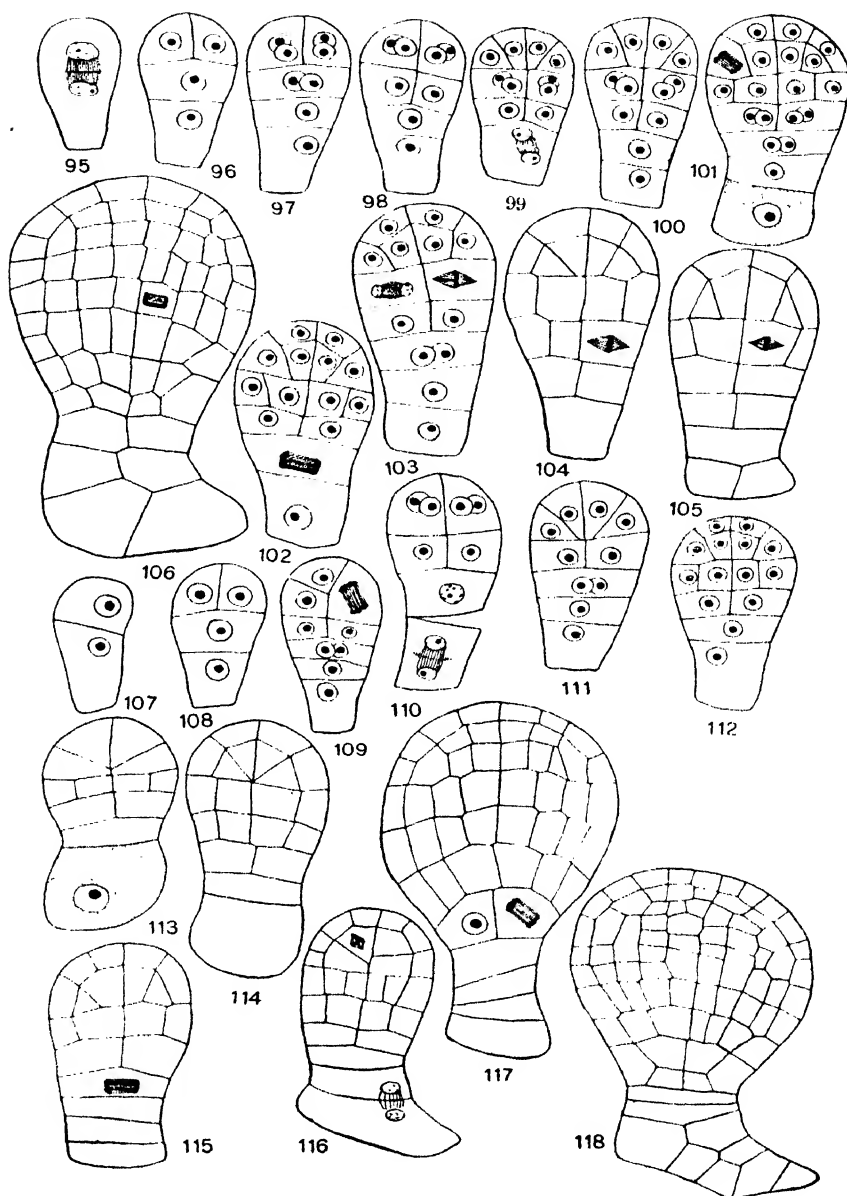
STERILITY AND UNFERTILIZED OVULES

In *Malvaviscus arboreus* Cav. studied by the writer (1954a), the flowers are sterile and drop off without setting seed. The embryo sacs are normally formed,



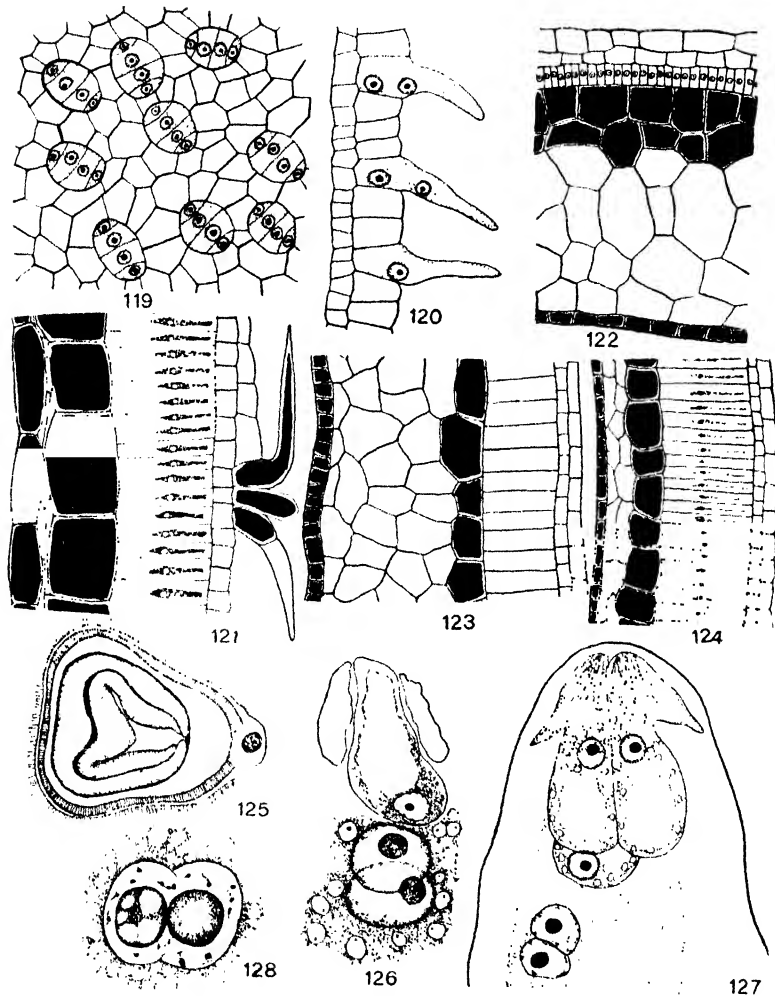
FIGS. 72-94.

- FIGS. 72-81. *Malachra capitata*. FIGS. 72-78. Stages in development of the embryo, $\times 340$.
 Fig. 79. Mature embryo, $\times 100$. Fig. 80. Ovule with a giant unfertilized embryo sac, $\times 100$. Fig. 81. Part of the embryo sac in the above magnified, $\times 205$.
 „ 82-94. *Hibiscus solandra*. FIGS. 82-92. Stages in embryogeny, $\times 340$. Fig. 93. Embryo with cotyledon primordia, $\times 230$. Fig. 94. L.S. mature seed, $\times 10$.



FIGS. 95-118.

FIGS. 95-106. Various stages in the embryo development of *Hibiscus hirtus*. $\times 320$.
 „ 107-118. Stages in the embryogeny of *Hibiscus micranthus*. $\times 320$.



FIGS. 119-128.

- FIGS. 119-125. Development and structure of seed coats in Malvaceae. Fig. 119. Surface view of the testa of *Hibiscus micranthus*, $\times 230$. Fig. 120. Development of hairs from testa of *H. micranthus*, $\times 230$. Fig. 121. Seed coats of *H. micranthus*, $\times 230$. Fig. 122. Seed coats of *Hibiscus solandra*, $\times 160$. Fig. 123. Seed coats from young seed of *Sida cordifolia*, $\times 160$. Fig. 124. Seed coats of *S. veronicaefolia*, $\times 160$. Fig. 125. T.S. seed of *Hibiscus micranthus*, $\times 10$.
- „ 126-128. Unfertilized embryo sacs. Fig. 126. Part of the unfertilized embryo sac of *Pavonia zeylanica*; note starch grains in cytoplasm, $\times 570$. Fig. 127. Upper part of an unfertilized embryo sac of *Sida cordifolia*; note starch grains in cells of egg apparatus, $\times 340$. Fig. 128. Hypertrophied polar nuclei from an unfertilized embryo sac of *Hibiscus hirtus*, $\times 570$.

but after reaching the 8-nucleate stage, the sacs as well as the ovules degenerate. In *Kydia calycina* the embryo sacs and ovules of the male flowers degenerate. In *Thespesia populnea*, a large number of flowers drop off without setting seed. The few fruits that develop show only one or two seeds each, though the ovary contains about 20 ovules. In all species in which the seeds develop normally, sometimes one or more ovules in any ovary may remain unfertilized. These ovules appear normal and occur along side with seeds containing well developed embryos (Figs. 80, 81). The cells of their integuments and nucellus remain intact but are devoid of cytoplasm. The embryo sac on the other hand enlarges a good deal, but continues to show normal contents. In one embryo sac of *Pavonia zeylanica* the synergids showed signs of degeneration but the egg and polar nuclei were still intact. Starch grains, normally absent from the embryo sac of this species, were present in this case (Fig. 126). In the fertilizable embryo sac of *Sida cordifolia*, starch grains are absent; but these were seen in the cells of the egg apparatus of an unfertilized embryo sac (Fig. 127). In *Hibiscus hirtus*, the polar nuclei in one unfertilized embryo sac became very large and hypertrophied; they show scanty chromatin and their nucleoli were vacuolated (Fig. 128). These instances might indicate a case of parasitism of an aggressive type of the female gametophyte on the sporophytic tissues in its struggle for survival. Stenar (1925) also noticed such unfertilized ovules in several species he investigated.

SUMMARY

Fertilization, development of the endosperm, embryo and seed have been studied in *Sida cordifolia* L., *S. veronicaefolia* L. (= *S. humilis* Willd.), *Pavonia zeylanica* L., *Abutilon indicum* G. Don., *Malackra capitata* L., *Hibiscus solandra* L. Herit., *H. hirtus* L. and *H. micranthus* L.

Pollen grains germinate quickly on the hairy stigma and produce pollen tubes in a polysiphonous manner. They penetrate through the well marked transmitting tissue of the style and enter the ovule in a porogamous manner. Their entry is facilitated by a hairy obturator in *Abutilon indicum* and *Hibiscus solandra* and a knob-like one in *Sida veronicaefolia*. The pollen tubes are 15–20 μ wide and persistent; their structure suggests that they might be useful in the transport of nutrients to the growing embryo.

The male gametes show distinct nucleoli. Formation of the primary endosperm nucleus occurs within 10–12 hours of pollination and its first division in about 24 hours in *Hibiscus solandra*. The endosperm which is nuclear becomes cellular at a comparatively late stage, by a process of indentation. Seeds are either non-endospermic or provided with a little endosperm. They lack perisperm.

Embryo development occurs according to the *Urtica* variation of the Asterad Type. The terminal cell of the two celled proembryo forms the cotyledons and stem tip; the basal cell gives rise to the hypocotyl, root tip, root cap and suspensor. The suspensor is large and boot-like.

The outer integument forms a membranous testa. Epidermal hairs develop in *Hibiscus micranthus* and *H. hirtus* while in *H. solandra* and *Pavonia zeylanica*, the cells become only papillate. The outer epidermis of the inner integument forms the palisade layer which shows well marked light line. The next one or two layers of cells constitute the pigment layer and the innermost, the fringe tissue. The median parenchymatous layers get crushed in the mature seed.

ACKNOWLEDGEMENTS

The writer wishes to express his grateful thanks to Dr. A. C. Joshi and Prof. J. Venkateswarlu for their kind interest in the work. His thanks are also due to Prof. P. Maheshwari for kindly going through the manuscript and to Prof. S. Rangaswamy for kindly translating Stenar's article from German.

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STUDIES ON THE NUTRITIVE VALUE OF PLANT PROTEINS: PART I.

PULSE PROTEINS—THEIR IMPROVEMENT BY AMINO ACID SUPPLEMENTATION *

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(Communicated by U. P. Basu, F.N.I.)

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INTRODUCTION

Studies on the investigations regarding the protein contents of seven important pulses, their digestibility, biological and growth-promoting values under raw and autoclaved conditions (Esh and Som, 1952) and some of their essential amino acid compositions (Esh and Som, 1953, 1954) have been reported in previous communications. These studies along with others (Ratnaprabha and Magar, 1953; Vijayaraghavan and Srinivasan, 1953) tend to indicate that some of these pulse proteins are not only associated with trypsin inhibitors affecting their nutritive value but all of them are deficient in methionine and/or tryptophan when compared to a standard protein (say, egg protein). Significant growth responses were, therefore, observed when these pulses were autoclaved and supplemented with methionine and fed to growing rats as an exclusive source of protein (Esh and Som, 1952; Ratnaprabha and Magar, 1953). Finding that such growth responses are still sub-optimum, attempts were made to increase further if possible the biologic utilization of these proteins by supplementing with other essential amino acids.

Two of the pulses, namely Bengal gram and lentil, which are deficient in sulphur containing amino acids and tryptophan, were taken for this purpose. Since the limiting amino acids as suggested by Mitchell and Block (1946) are likely to improve the protein utilization, the pulses were first studied after supplementing with methionine and/or tryptophan. In view of the observations that the utilization of rice protein (Pecora and Hundley, 1951), wheat protein (Sure, 1952) and groundnut protein (Esh and Basu, 1954) is significantly increased when supplemented with threonine indicating that the amino acids which are 'limiting' chemically may not be always so physiologically, experiments were conducted also after supplementing these pulses with threonine.

EXPERIMENTAL

The pulses Bengal gram and lentil obtained from the market and ground to 40 mesh were used after autoclaving at 15 lbs. pressure for 30 minutes in all these experiments. The protein efficiency of these pulses (gain per gram of protein eaten) was measured by the rat growth method by feeding weanling litter mate rats diets containing equal suboptimal amounts of proteins (12%) provided by the respective pulses. The basal diet contained in grams, hydrogenated fat 9, salt mixture (U.S.P. XIV) 4, cod-liver oil 2, choline chloride 0.2, sufficient autoclaved pulse powder to maintain 12% protein level and corn starch to make 100 grams. 100 grams of this diet was supplemented with 1 c.c. vitamin B mixture † and 0.5 c.c. of vitamin

* Based on the paper read at the Symposium on 'Proteins in health, disease and industry' held in August, 1954, under the auspices of the National Institute of Sciences of India.

† Thiamine hydrochloride 20 mg., riboflavin 30 mg., nicotinic acid 200 mg., pyridoxine hydrochloride 12.5 mg., panthenol 150 mg., folic acid 5.0 mg. and biotin 0.5 mg. dissolved in 50 c.c. alcohol.

K and E mixture.* The essential amino acid contents of both the pulses as analysed by chemical and microbiological methods (methionine, tryptophan, lysine, threonine and phenylalanine chemically and leucine, isoleucine and valine microbiologically) have been tabulated in Table I, with those of casein and egg protein as collected from Block and Bolling (1945).

TABLE I

*Essential amino acid composition of Bengal gram and lentil.
(Calculated on the basis of 16% nitrogen)*

Amino Acid	Egg	Bengal gram	Lentil	Casein
Methionine ..	4.1	1.2	0.6	3.5
Tryptophan ..	1.5	0.75	0.6	1.2
Lysine ..	7.2	6.3	5.1	7.9
Threonine ..	4.9	2.8	3.0	4.1
Phenylalanine ..	6.3	4.9	3.98	5.6
Leucine ..	9.2	8.2	5.50	9.9
Isoleucine ..	8.0	6.5	5.80	6.5
Valine ..	7.3	5.5	5.1	6.7
Arginine ..	6.4	6.9†	6.9†	4.2
Histidine ..	3.0	2.3†	2.1†	2.1

The approximate amounts of the essential amino acids supplied by the pulses at the level used (12%) in these experiments are placed in Table II. In this table also is shown the order of deficiency of the essential amino acids in pulses as calculated from their amino acid composition and the minimum daily amino acid requirements for the growing rats as reported by Rose (1937). For comparative

TABLE II

The order of deficiency of the essential amino acids in pulses when compared with daily requirement for growth

Amino acids	Requirement for growth % of diet	Bengal gram		Lentil		Casein	
		Supplied in the diet	Deviation from requirement	Supplied in the diet	Deviation from growth	Supplied in the diet	Deviation from growth
		%	%	%	%	%	%
Methionine ..	0.6	0.14	-76	0.072	-88	0.42	-30
Tryptophan ..	0.2	0.09	-55	0.072	-64	0.15	-25
Lysine ..	1.0	0.76	-24	0.61	-39	0.95	-5
Threonine ..	0.5	0.33	-34	0.36	-28	0.5	0
Histidine ..	0.4	0.25	-37	0.25	-37	0.25	-37
Phenylalanine ..	0.7	0.59	-15	0.48	-30	0.67	-3
Valine ..	0.7	0.66	-6	0.60	-14	0.79	+12
Leucine ..	0.8	0.98	+22	0.66	-17	1.18	+47
Isoleucine ..	0.5	0.78	+56	0.69	+38	0.78	+56
Arginine ..	0.2	0.82	+300	0.82	+300	0.5	+150

* Menadion (vitamin K) 20 mg. and alpha-tocopherol 1,000 mg. dissolved in 100 c.c. arachis oil.

† Calculated from the results reported by Vijayaraghavan and Srinivasan (1953).

studies, therefore, different groups of rats were fed experimental pulse diets after supplementation with most deficient amino acids making approximate allowance for the inactive forms. The slight variation in total nitrogen contents of the diets due to such supplementation was, however, compensated by varying the pulse and starch contents. Weanling albino rats as raised in our own colony and weighing about 40-45 gms. were divided among the groups with usual precautions as to distribution of litters, sex and weight. They were housed individually in screen bottom metal cages with food and distilled water *ad libitum*. Groups receiving a standard diet containing casein in the same level were included in each set of experiments as a guide for establishing relative rates of growth. All these tests were continued for three weeks. Animals were weighed twice in a week and total food intake of each animal was recorded.

A second set of experiments was designed with enzymic digests of these pulses both before and after supplementation with deficient amino acid. The pulses were digested with papain and the protein hydrolysates thus obtained were fed to protein depleted adult rats as the sole source of protein in order to find the nature of protein regeneration during the repletion phase particularly after supplementation with deficient amino acids.

RESULTS AND DISCUSSION

The results of feeding Bengal gram diet at 12% protein level both with and without amino acid supplementation are presented in Table III. As previously obtained (Esh and Som, 1952), methionine supplementation made significant growth increment over that obtained with the pulse only. It is interesting to find that single supplementation with other deficient amino acids like tryptophan, threonine or lysine could not induce greater growth. But when supplemented with these amino acids in presence of methionine, only threonine could enhance significant growth response beyond that obtained after supplementation with methionine

TABLE III

Average growth increments in rats when Bengal gram diets were fed for 3 weeks after supplementation with deficient amino acids. (Average of six rats used in each group)

Diet	Wt. gain in gm.	Gain increase %	Food intake in gm.	Protein intake in gm.	Protein efficiency ratio
Bengal gram ..	24.1 ± 0.6*	..	155.3	18.6	1.29
.. .. + methionine ..	32.3 ± 0.8	34.1	160.7	19.3	1.67
.. .. + tryptophan ..	22.0 ± 1.5	0	152.0	18.2	1.21
.. .. + threonine ..	24.8 ± 1.7	0	148.2	17.8	1.39
.. .. + lysine ..	24.0 ± 1.2	0	155.0	18.5	1.30
.. .. + methionine and tryptophan.	35.1 ± 2.1	45.6	156.4	18.8	1.87
.. .. + methionine and threonine.	44.5 ± 1.5	84.6	162.0	19.4	2.29
.. .. + methionine and lysine.	32.8 ± 0.9	36.0	156.0	18.7	1.22
.. .. + methionine, threo- nine and trypto- phan.	48.3 ± 1.2	100.4	172.1	20.7	2.34
.. .. + methionine, threo- nine, tryptophan and lysine.	47.9 ± 2.1	100.0	170.0	20.4	2.35
Casein	50.5 ± 1.8	109.5	178.6	21.4	2.36

* Standard error of the mean calculated by the formula $\sqrt{\frac{\sum(x - \bar{x})^2}{n(n-1)}}$

only. The slight increment with tryptophan in presence of methionine is of doubtful significance although in presence of methionine and threonine it could induce a little greater growth activity. The addition of lysine with the group methionine, threonine and tryptophan could not enhance further growth. The rats of all the comparable groups have taken significant quantity of food and the data for protein efficiency ratio as calculated tend to indicate that the efficiency of protein utilization has significantly increased when the diet was supplemented with methionine and threonine. Apparently two conclusions may be arrived at that while the supplementation with the most limiting amino acid methionine can improve the nutritive value of the pulse protein, other deficient amino acids fail to do so in absence of methionine but threonine in presence of methionine can accelerate growth activity of pulse protein. Actually how these added amino acids function cannot be explained at this stage of our knowledge.

Similar experiments have been conducted with lentil protein and the results are presented in Table IV. In this case also maximum growth increment was obtained when the diet was supplemented with the most deficient amino acid—methionine and supplementation with other deficient amino acids has practically no effect.

TABLE IV

Growth increments in rats when lentil diets (at 12% protein level) were fed for 3 weeks after supplementation with deficient amino acids. (Average of 6 rats used in each group.)

Diet	Wt. gain in gm.	Gain increase %	Food intake in gm.	Protein intake in gm.	Protein efficiency ratio
Lentil diet ..	12 ± 1.1 *	..	149.5	18.0	0.67
.. + methionine ..	33.25 ± 1.8	177	161.5	19.38	1.72
.. + tryptophan ..	11.9 ± 1.9	..	148.0	17.8	0.67
.. + lysine ..	12.1 ± 1.8	..	150.5	18.1	0.67
.. + threonine ..	11.7 ± 2.1	..	151.0	18.12	0.64
.. + methionine and tryptophan.	37.5 ± 1.8	212	153.3	18.4	2.04
.. + methionine and threonine.	36.2 ± 2.1	202	173.3	20.8	1.74
.. + methionine and lysine	32.8 ± 1.1	175	158.0	18.96	1.71
.. + methionine, tryptophan and threonine.	50.2 ± 2.5	318	161.0	19.34	2.59
.. + methionine, tryptophan, threonine and lysine.	49.07 ± 2.2	309	156.7	18.81	2.60

* Standard error of the mean calculated by the formula $\sqrt{\frac{\sum(x - \bar{x})^2}{n(n-1)}}$.

In presence of methionine, however, while addition of either tryptophan or threonine has induced almost equal growth stimulation (in case of tryptophan a little greater) curiously enough, their incorporation together has influenced the growth activity to a significantly higher level raising the protein efficiency ratio from 0.67 (pulse only) to 2.59. Addition of the next deficient amino acid lysine failed to improve the nutritive value further.

The results obtained with enzymic digest of lentil when fed to protein depleted rats are placed in the Graph I, below.

The nature and degree of growth increments observed during the repletion phase when the different diets were fed to the protein depleted rats tend to indicate the same trend of results when the intact pulse was fed after supplementation with the deficient amino acids.

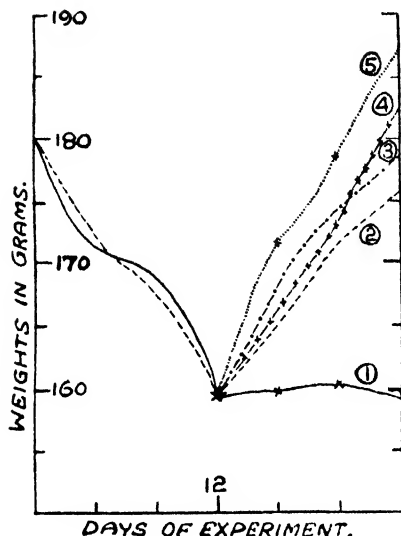


FIG. 1. Weight regeneration of protein-depleted adult rats when an enzymic digest of lentil (5% protein solution) was fed as an exclusive source of protein for 12 days. (1) Lentil hydrolysate, (2) 1 with methionine, (3) 1 with methionine and tryptophan, (4) 1 with methionine, tryptophan and threonine, and (5) casein hydrolysate.

The results as reported above tend to show that although supplementation with most deficient amino acid, methionine, increases the growth-promoting capacity of the pulses significantly, additional growth was obtained when tryptophan and threonine were added with methionine. Thus with Bengal gram, methionine has raised the protein efficiency ratio from 1.29 to 1.67 and methionine along with tryptophan and threonine the same has been raised to 2.34, about 100% improvement over the basal diet. With lentil also while methionine has enhanced the protein efficiency ratio from 0.67 to 1.7, addition of threonine and tryptophan has raised the same further to 2.6 about 50% improvement over the value obtained by methionine supplementation only. The similar results obtained with enzymic digest of lentil after amino acid supplementation give testimony to the fact that the same pattern of amino acids as obtained from the pulses and as modified by the addition of deficient amino acids is effective in promoting growth as well as in regenerating weight loss when fed to the protein-deficient animals.

Further work is in progress regarding the influence of supplementing the pulse proteins with other protein foods rich in methionine, threonine and tryptophan on their growth-promoting value.

SUMMARY

Improvement on the nutritive value of pulses, Bengal gram and lentil was effected by supplementing them with deficient amino acids—methionine, tryptophan and threonine. It has been observed that when these pulses were fed at 12% protein level, supplementation with most deficient amino acid methionine significantly enhanced their growth-promoting capacity. Supplementation with other deficient amino acids like tryptophan, lysine or threonine individually in absence of methionine was ineffective, but in presence of methionine, tryptophan and/or threonine significantly enhanced the nutritive value further. Thus maximum improvement was obtained with the combination of methionine, tryptophan and threonine raising the protein efficiency ratio of Bengal gram from 1.29 to 2.34 that of lentil from 0.67 to 2.59.

The same trend of improvement of the pulses was observed when an enzymic digest of the pulse lentil was fed to protein-depleted adult rats for regeneration of growth.

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MORPHOLOGY AND HISTOLOGY OF THE AIR-BLADDER OF CERTAIN SCIAENOID FISHES WITH THE DESCRIPTION OF A NEW TYPE OF EAR-AIR BLADDER CONNECTION *

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INTRODUCTION

In the fishes of this family, whose musical performances probably have given rise to the Homeric fable of the song of the sirens, the air-bladder, with a few exceptions, show the most extraordinary development of short ramified caeca on the sides and at the two extremities. Due to these interesting developments the present author worked on some of the Indian sciaenoid fishes and has come across an altogether new type of ear-air-bladder connection.

MATERIAL AND TECHNIQUE

The family is circumtropical in distribution and some of the forms are found even in the temperate seas. In the Indian rivers they ascend up to very good distances and have been caught at places 600 miles from the sea. *Sciaena coitor* (Ham.) and *Pama pama* (Ham.) are found locally at Allahabad, whereas *Sciaena albidu* (Day) and *Sciaena miles* (Cuv. & Val.) were collected at Bombay.

Fishes were dissected along the mid-ventral line and the bladders were first fixed in situ and then removed from the fish. The fixative generally used was Bouin's picro-formal-acetic fluid. The sections were mostly treated with Delafield's haematoxylin counterstained with 0.5%-1% alcoholic eosin.

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OBSERVATIONS ON *SCIAENA COITOR* (HAM.)

A. *External Morphology*

The air-bladder (Plate IX, fig. 1) is large (about one-third of the total length of the fish), shaped like a bag and pointed behind. In a fish measuring 7 inches in length the air-bladder was found to be 2.5 inches long. The anteriormost part of the bladder bulges out laterally in a transverse manner to the rest of the bladder. The pointed posterior region ends in the vicinity of the anal opening.

On either side the air-bladder is furnished with ten to eleven short caeca (Plate IX, fig. 1, *Cae*) which are sub-divided into smaller and smaller branches and are surrounded by voluminous lobes of fat. A few caeca found in the hindmost part of the air-bladder may sometimes remain undivided. The cavity of the air-bladder continues in these caeca as well (Plate X, fig. 3) which contains the same gas as the air-bladder. A cluster of caecum of one of the anterior branches ends under the fine transparent skin of the opercle. In the region of the head, the caeca enveloped by the fat and conjunctiva tissue and the cephalic prolongation, need a very careful dissection in order to separate them. Some of the caeca of the head region are connected with the auditory organs and this condition is described presently. The bladder is of the physoclistous type.

B. *Histology*

The wall of the air-bladder is quite thick, the outer membrane being fibrous and very compact and presents a shining silvery white hue. The wall has got two distinct layers (Plate X, fig. 3, *Tu.ex.*, *Tu.in.*)—(i) Tunica externa and (ii) Tunica interna. The tunica externa is formed by elastic and non-muscular fibres. The tunica interna usually consists of a layer of conjunctiva tissue which lodges the blood capillaries.

C. *The Gas-gland*

It is situated on the ventral wall of the anterior region of the air-bladder and can be noticed after opening the bladder cavity (Plate IX, fig. 1, *G.Gl.*). It consists of a mass of many layers of cells forming a massive glandular epithelium (Plate X, fig. 1, *G.Gl.*). The rete mirabile is always continuous with the glandular epithelium (Plate X, fig. 1, *RM.*). The blood capillaries of the rete do not join before supplying the gland and are thus of the type of rete mirabile unipolar duplex. Occasionally we find definite spaces as gaps in the gas gland (Plate X, fig. 2, *G.gl.L.*).

D. *The Oval*

Situated on the dorsal side of the air-bladder opposite the gas-gland, we find the opening of the oval (Plate IX, fig. 1, *OV.*). This organ of the air-bladder is meant for removing gas from the air-bladder and is functionally analogous with the pneumatic duct of the physostomous fishes. In the wall of the oval we find numerous blood capillaries, which at the time of need absorb the gas of the air-bladder. Round the opening of the oval we find circular muscle fibres and a few longitudinal ones as well which control the opening like sphinctor muscles. The name 'oval' was first given by Corning (1888) due to the characteristic shape of its opening.

E. Ear-Air-Bladder connection

From the anterior region of the air-bladder arise two caeca each of which are again sub-divided into three groups of smaller caeca. A cluster of these caeca proceeds upwards and forwards by the side of the corresponding auditory capsules which has on its anterior side an opening closed by a membrane (Plate IX, fig. 1). The caecal prolongation of the air-bladder comes into contact with the membrane on the outer side which on the inner side has the perilymph fluid surrounding the internal ear.

OBSERVATIONS ON *PAMA PAMA* (HAM.)

The air-bladder is a very prominent structure in the cavity beneath the vertebral column. In a fish 8.5 inches long, the air-bladder was found to be 3.9 inches in length and the maximum width which is found in the anterior region being 0.8 inches (Plate IX, fig. 2, *AB*). The bladder has a shining silvery appearance and gradually tapers posteriorly till it becomes pointed and extends to a short distance beyond the anal opening. From the hindmost part of the air-bladder arise two narrow tubes (Plate IX, fig. 2, *Tub*) which proceed anteriorly without dividing. At the point where the bladder ends anteriorly the tubes divide into a number of caeca (Plate IX, fig. 2, *Cae*) and one of the caecum on either sides proceeds upwards and becomes connected with the auditory organs just as in the case of *S. coitor* (Ham.) with the only difference that in *Pama pama* (Ham.), only one caecum becomes connected with the membrane on the anterior side of the auditory capsule, whereas in *S. coitor* (Ham.) a cluster of caeca get attached. The air-bladder is of physoclistous type.

The bladder wall is very thick and but for this, the histological structures of the bladder wall, the gas-gland, the rete mirabile and the oval are just similar to that of *S. coitor* (Plate X, figs. 4, 5). The position of these organs are also identical in both the fishes.

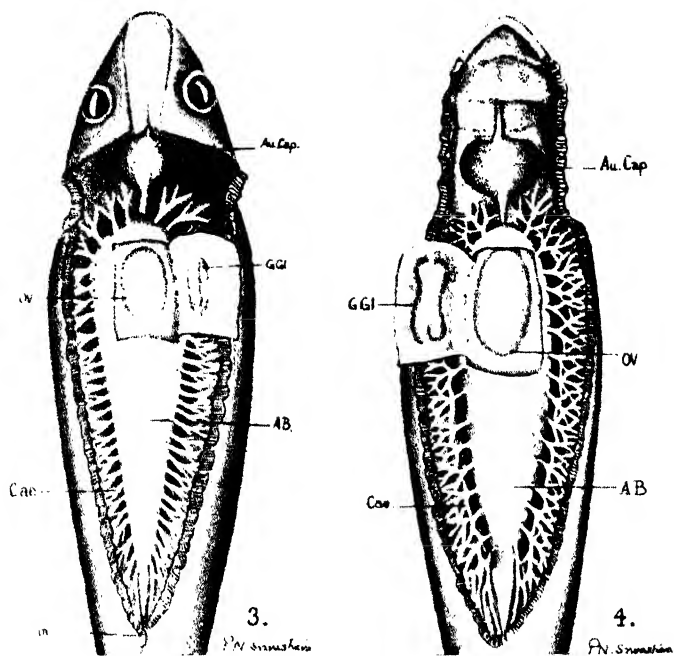
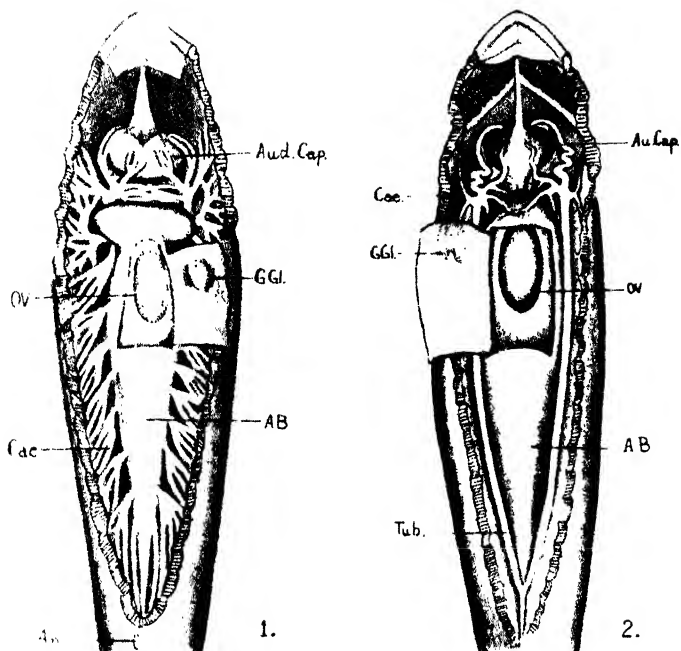
OBSERVATIONS ON *SCIAENA ALBIDA* (DAY)

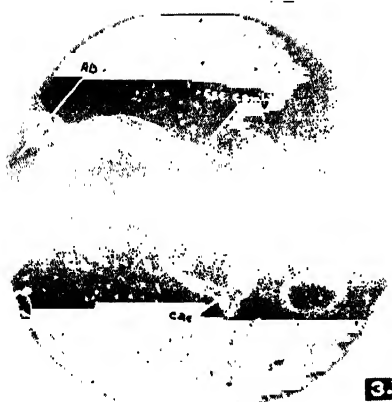
The air-bladder of *S. albida* is somewhat similar to *S. coitor* with a few differences. The bladder is shining and is 2.2 inches in length in a fish measuring 6.3 inches (Plate IX, fig. 3, *AB*). Posteriorly it tapers and ends in the vicinity of the anal opening. On the sides, the bladder is furnished with twenty-seven caecal outgrowths of a simple nature (Plate IX, fig. 3, *Cae*). The lumen of the air-bladder is continuous with that of the caeca. The most important difference with *S. coitor* is that in this case the caecal outgrowths do not proceed forwards and as such the ear-air-bladder connection is wanting.

The position of the gas-gland and the Oval (Plate IX, fig. 3, *G.Gl, OV*) are the same as in *S. coitor* (Ham.). Histologically also the gas-gland shows the same type of glandular epithelium and the rete is of the type of rete mirabile unipolar duplex (Plate X, fig. 6).

OBSERVATIONS ON *SCIAENA MILES* (CUV. & VAL.)

The air-bladder of *Sciaena miles* has the most shining colour than those seen in the preceding species. In a fish 8.2 inches long the air-bladder measures 2.7 inches in length, the maximum width being 0.75 inches (Plate IX, fig. 4, *AB*). Posteriorly this bladder also tapers down and ends in the vicinity of the anal opening. From the sides of the air-bladder are given off 15-16 caecal outgrowths (Plate IX, fig. 4, *Cae*) which branch dicotomously and are not simple as in *S. albida* and *S. coitor*. These outgrowths are more prominent. As in the other sciaenoid fishes the caeca are covered with fat. The ear-air-bladder connection is lacking as in *S. albida*.





The shape and position of the Oval and the gas-gland are almost similar to those of *S. coitor* and *S. albida* (Plate IX, fig. 4, *OV*; *G.Gl.*).

DISCUSSION ON THE NEW TYPE OF EAR-AIR-BLADDER CONNECTION

Weber (1820) while working on five groups of fishes, described three types of ear-air-bladder connection differing entirely from one another in anatomical characters. These three types of connections are as follows:—

First type: It is essentially in a relation of simple apposition of a precoelomic diverticulum of the air-bladder to the base of the auditory capsule. It is present in Notopterus, Sparidae, and some species of Serranidae and Gadidae.

Second type: In this case, the anterior diverticulum of the air-bladder, in the form of minute capillary tube, extends into the head on each side, and ends in two large expanded vesicles which occupy an extensive and complicated cavity in the bones of the lateral and basilar region of the skull. This type of connection is found in Clupeidae.

Third type: This type of connection is affected by means of an articulated chain of small bones developed from the anterior vertebrae which serve to connect the anterior end of the air-bladder with the perilymph cavity. This type is known as 'Weberian mechanism' and is found in the families Cyprinidae, Siluridae, Characinae and Gymnoti.

Besides these, I came across a connection of ear and air-bladder in *Sciaena coitor* (Ham.) and *Pama pama* (Ham.) belonging to the family Sciaenidae. In these cases certain caeca arise from the side of the air-bladder and some of them proceed anteriorly towards the auditory capsule which has got an oval opening on its anterior side closed by a membrane. The caecal prolongation of the air-bladder comes into contact with this membrane on the outer side while on its inner side lies the perilymph fluid surrounding the membranous labyrinth of the ear. This type of connection falls in altogether a different category than those described by Weber and as such it should be regarded as a fourth type of connection between the ear and the air-bladder.

SUMMARY

The air-bladder in *Sciaena coitor* (Ham.), *Pama pama* (Ham.), *Sciaena albida* (Day) and *Sciaena miles* (Cuv. & Val.) are large and about one-third of the total length of the fish, shaped like a bag and pointed behind. All of them except *Pama pama* (Ham.) are furnished with numerous short caecal outgrowths which are surrounded by fat. In *Pama pama* (Ham.) only two outgrowths arise from the sides of the air-bladder from the hindmost region which proceed upwards and divide anteriorly. The gas-gland, and oval are well developed in all the cases. *Sciaena coitor* (Ham.) and *Pama pama* (Ham.) show the new type of ear-air-bladder connection, the like of which has not yet been recorded in any fish. This connection is wanting in *Sciaena albida* (Day) and *Sciaena miles* (Cuv. & Val.).

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ABBREVIATIONS USED

AB—Air-bladder; *An*—Anal opening; *Au.Cap*—Auditory capsule; *Cae*—Caecal Outgrowths; *G.Gl*—Gas gland; *G.gl.L.*—Gas gland Lumina; *RM*—Rete mirabile; *Tub.*—Tubular outgrowth of the air-bladder; *Tu.ex*—Tunica externa; *Tu.in*—Tunica interna; *OV*—Oval.

EXPLANATION OF PLATES

PLATE IX

- FIG. 1. *Sciaena coitor* dissected ventrally to show the air-bladder in situ, showing the positions of the gas gland and oval; connection of the air-bladder with the auditory capsule can be seen.
- „ 2. *Pama pama* dissected ventrally to show the air-bladder in situ. Two tubes are seen arising from the posterior end of the air-bladder. Positions of gas gland, oval and the air-bladder auditory capsule connection can be seen.
- „ 3. *Sciaena albida* dissected ventrally to show the disposition of the air-bladder in situ. Red gland and the oval can be noticed. There is no connection with the ear.
- „ 4. *Sciaena miles* dissected ventrally to show the air-bladder in situ. Caecal outgrowths are dicotomously branched. Oval and the gas gland are seen opposite each other. Connection between the air-bladder and the ear is lacking.

PLATE X

- FIG. 1. *Sciaena coitor*. Section showing the prominent retia mirabilia and gas-gland together with the two layers of the air-bladder wall.
- „ 2. *Sciaena coitor*. Sections showing the bladder wall, retia mirabilia and gas-gland lumina which appears as clear gap.
- „ 3. *Sciaena coitor*. Section passing through the air-bladder and caecal outgrowths showing that the cavity of the air-bladder is continuous with that of the caeca. The two layers of the bladder wall can be very clearly seen.
- „ 4. *Pama Pama*. Section passing through the gas-gland and rete mirabile. Blood vessel supplying the rete can be noticed.
- „ 5. *Pama pama*. Section showing the bladder wall, gas-gland, rete mirabile and the gland lumina as clear gap.
- „ 6. *Sciaena albida*. Section showing the two bladder walls, gas-gland and the rete mirabile.

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SOME OBSERVATIONS ON PLANTS OF THE SOUTH INDIAN HILLTOPS AND THEIR DISTRIBUTION *

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It is a well-known fact that hilltops in the tropics possess a characteristic flora of their own. Generally there is a change in the aspect of the vegetation at about 3,000 feet (1,000 metres) altitude, as shown by Trimen (1886) for Ceylon, Meebold (1909) for Bababudangiris in Mysore, Stamp (1925) for Burma, Ward (1927) for Sino-Himalayas, and Steenis (1934-36) for Malaysia.

Chatterjee (1940) while studying the endemic flora of India and Burma observes that in the hilly or mountainous regions—in the Himalayas, Khasias, Burma hills and Nilgiris, altitude is the dominating factor in determining the nature of vegetation. It may be remarked that although these regions are widely separated, the vegetation of the upper subtemperate regions is similar in all. In all cases the lower zone is characterized by evergreen forests, and the higher altitudes tend to have subtemperate forests as evidenced by the larger number of species adapted to life in cooler climates. Thus there is a transition from the evergreen to the subtemperate species as one ascends the hills. This transition has been shown by Steenis (1934-36) to occur at 1,000 m., and has been observed by the present author in the vegetation of Nandidroog, Bababudangiris and Biligirirangans of Mysore State, the Pulneys and Nilgiris in Madras State, and the hills in Poona District (like Simhagad and Torna) of Bombay State. Here the plants are exposed for the greater part of the year to cool climates and the diurnal range of temperature is not great. Whereas in the evergreen forests at the base of these hills humidity is the master factor, in the higher altitudes temperature is important.

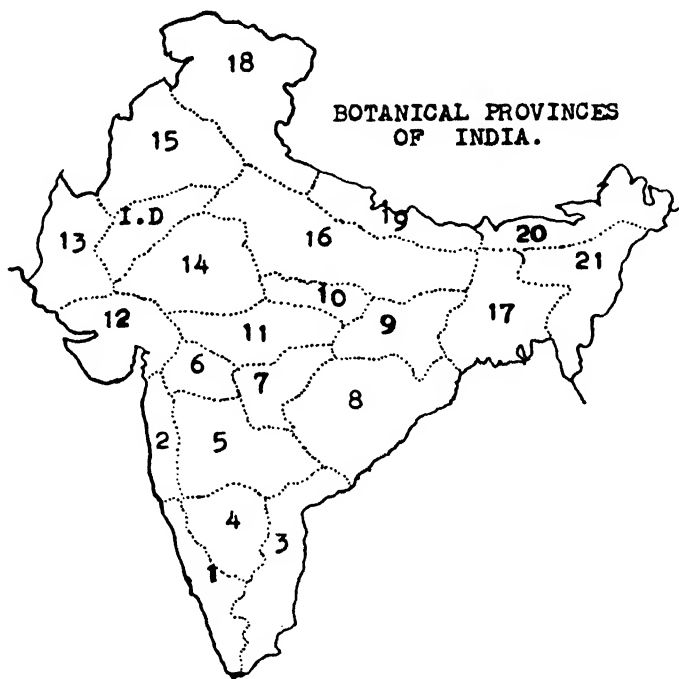
In the present study an attempt has been made to find out the status of the subtemperate species of the South Indian hilltops, and, from their distribution, determine the origin or origins of this flora. With this end in view, a list of all plants reported from above 3,500' in the South Indian hilltops of Madras and Mysore States has been prepared from available literature, and their present distribution checked by reference to all floras of the adjacent regions.

For an analysis of the distribution of hilltop plants it becomes necessary to consider the botanical provinces of India. Pioneer work on dividing India into such provinces was done by Hooker and Thomson (1855); and later workers on the same include Clarke (1898), Prain (1903-08), Hooker (1909), Calder (1938) and Chatterjee (1940). Among these, Hooker and Thomson's divisions, which are based on physiographic and climatic factors as affecting growth and distribution of vegetation are the only ones which can be utilized for a detailed analysis of distribution of plants within India. The others' divisions are far too generalized to be of any use for this purpose. Hence, the provinces of Hooker and Thomson (1855, pp. 118-245) are utilized for the present study. The approximate outlines of these provinces are shown on the accompanying map, the provinces being serially

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numbered for easy reference. In this account, 'India' is understood to mean the whole of the sub-continent excluding Burma. The only deviation from Hooker and Thomson is that their East India has been replaced by North-east India and includes Assam, Cachar, Silhet, Chittagong, Tippera, Mishmee, Naga and Khasia hills. The other provinces are as defined by Hooker and Thomson (*l.c.*).



These provinces are: (1) Malabar (Ceylon is included here for the sake of the present study); (2) Concan; (3) Carnatic; (4) Mysore; (5) Deccan; (6) Khandesh; (7) Berar; (8) Orissa; (9) Bihar; (10) Bandelkhand; (11) Malwa; (12) Gujerat; (13) Sind; (14) Rajwara; (15) Punjab; (16) Upper Gangetic Plain; (17) Bengal; (18) North-west Himalayas; (19) Central Himalayas; (20) Eastern Himalayas; (21) North-east India; and I.D. = Indian Desert.

In the list referred to above, all species reported to occur above 3,500' altitude in the hills of Madras State and Mysore have been included. Thus, there is a large number of species which have their nearly related species occurring at lower altitudes in these hills, or themselves occur at lower altitudes elsewhere. The last category has been omitted. In analyzing the distribution of the hilltop plants the following points have been kept in view:—

(i) The total surface on which mountain genera can settle is extremely small, the area below 3,500' being cut off. Hence, this mountain flora can be compared to an island flora for all practical purposes, or, as Steenis (1934-36) says these hilltops can be compared to islands of subtemperate vegetation in a sea of tropical vegetation found at the base of these mountains.

(ii) The mountain flora is a more favourable subject for study than the true tropical flora of the lowlands, because the temperate elements of the mountain flora represent an offshoot from the centres of development of temperate genera.

(iii) Such a mountain flora has two types of plants: (a) representatives of genera which are common to and centre in temperate regions; (b) representatives of a true tropic stock which have produced temperate species.

(iv) By limiting our consideration to category (a) the number of possible explanations is reduced; while category (b) does not furnish critical evidence for former migrations, as it is understood that their representatives have originated where they are found at present.

(v) Proof of actual migration must be given. Since proof from fossils is not available to us, we have to search for it in the present distributional facts by considering the distribution of all the species of a genus, and by comparing their specific areas with one another.

(vi) Perhaps a less critical proof would be to compare distribution patterns of groups of species.

Taking methods envisaged in (v) and (vi) together, the question revolves itself in finding the distribution patterns of present day plants and their bearing on ancient dispersals.

(vii) That dispersal and migration of plants take place cannot be denied, since all plants extend their areas. Now, for each genus there seems to be a centre or centres of development. From the centre of development to the border, the number of species decreases. The number of species on the borders of genera will naturally show the direction of migration from the centre to the periphery, though not in all cases.

DISTRIBUTION PATTERNS

In accordance with the occurrence of hilltop species in various provinces as shown on the Map there are a few fundamental distribution patterns discernible. The following are the main patterns and their variations as exhibited by the 1,224 species so far reported from hills in Madras and Mysore States.

A. There are 857 species in Province 1, and a further 97 in Province 2. Thus there are 954 species restricted to the Western Ghats and extending to Ceylon. All these can be taken to be endemics and as such of not much value in giving us any direct clues as to the origins or routes of migrations. It should not, however, be forgotten that such endemics could be utilized for the purpose indicated.

B. Species that occur in Provinces 1, 2, 3 form one basic pattern. Here it should be remembered that their occurrence has been reported from the northern part of Province 3. This pattern obviously shows an intermigration of species between the Eastern and the Western Ghats. Alternatively, these species could have reached the Eastern and Western Provinces from some common source. Such species include:

Dysophylla myosuroides Benth.,
Elaeagnus kologa Schlecht.,
Eulophia ochreatea Lindl.,
Ipomaea wightii Choisy.,

Microstylis versicolor Lindl.,
Smithia hirsuta Dalz.,
Solanum denticulatum Bl.,
Viburnum acuminatum Wall.,
Wendlandia notoniana Wall.

Atylosia sericea is similar but does not occur in Province 1.

Species that occur in Provinces 1 and 3 include :

Adenostemma reticulatum DC.,
Amphilophis insculpta Stapf.,
Anaphalis lawii Gamble.,
Blumea hieracifolia DC., var. *macrostachya*
 Hook f.,

Bupleurum mucronatum W. & A.,
Carex myosurus Nees.,
Celosia pulchella Moq.,
Cinnamomum wightii Meissn.,
Decalepis hamiltonii W. & A.,

<i>Eriocaulon conicum</i> (Fyson), Fischer	<i>Lonicera leschenaultii</i> Wall.,
= <i>E. diana</i> Fyson., var. <i>conica</i> Fyson.	<i>Ophiorrhiza hispidula</i> Wt.,
<i>Eulalia phaeothryx</i> O. Kt. (Tonkin).	<i>Osbeckia hispidissima</i> Wt.,
<i>Exacum perottetii</i> Griseb.,	<i>Pavetta breviflora</i> DC.,
<i>Habenaria longicornu</i> Lindl.,	<i>Rungia parviflora</i> Nees., var. <i>monticola</i>
<i>Knoxia heyneana</i> DC.,	Gamble.,
<i>Ligustrum roxburghii</i> Clarke.,	<i>Strobilanthes cuspidatus</i> T. And.

Species in Provinces 1, 2, 3, 8 and 9 are :

Exacum bicolor Roxb.,
Solanum giganteum Roxb.,
Sopubia trifida Buch. Ham.

Species in Provinces 1, 2, 8 and 9 include :

Euphorbia pycnostegia Boiss.,
E. zornii Boiss.,
Habenaria digitaria Lindl., var. *foliosa* Hook f.

Species in Provinces 1, 2, 10 and 11 :

Senecio edgeworthii Hook f.

Species in Provinces 1, 2 and 9 include :

Eriocaulon conicum Hook f.,
Fimbristylis nigrobrunnea Thw. (Nicobars, Cambodia),
Justicia orbiculata Wall. (Siam),
Oxytenanthera nigrociliata Munro.,
Pilea trinervia Wt.,
Gymnema hirsutum W. & A.,
Sideroxylon tomentosum Roxb.

Species in Provinces 3, 8 and 9 include :

<i>Brachiaria kurzii</i> A. Camus.,	<i>Lasiococca comberi</i> Haines.,
<i>Hypserpe cuspidata</i> Miers.,	<i>Pygeum andersonii</i> Hook f.
<i>Lasianthus tomentosus</i> Bedd.,	

Species in Province 3 only :

Osbeckia reclava D. Don., var. *pulchella* Triana.,
Memecylon madgolense Gamble.,
Senecio candicans DC.,
Tephrosia roxburghiana J. R. Drumm.

Carissa paucinerva A. DC., occurs in Provinces 1, 3, 8 and 9.

From a perusal of the above distributions it is seen that there is a disjunction between the western and the eastern provinces. All the same one can discern a south-west to north-east trend in these disjunctions. This becomes more evident in the following distribution patterns.

C. Species that occur in Provinces 1, 2, 3, 8, 9, 18-21 :

Lageria alata Sch. Bip. (China, Burma, Malaya, Philippines, tropical Africa),
Rubus ellipticus Sm. (China, Burma).

Species in Provinces 1, 3, 8, 9, 18-21 :

- | | |
|---|---|
| <i>Artemisia parviflora</i> Buch. Ham., | <i>Polygonum chinense</i> Linn. (Burma, |
| <i>Campanula canescens</i> Wall. (Pegu), | China, Malaya, Japan), |
| <i>Conyza japonica</i> Less. (China, Burma, | <i>Rhamnus nepalensis</i> Lows., |
| Afghanistan), | <i>Symplocos spicata</i> Roxb. (China, Japan, |
| <i>Exacum tetragonum</i> Roxb. (China), | Martaban), |
| <i>Hydrocotyle rotundifolia</i> Roxb. (Malaya), | <i>Thalictrum javanicum</i> Bl., |
| <i>Laggera pterodonta</i> Roxb. (Burma, | <i>Viola patrinii</i> DC. (Afghanistan, W. |
| tropical Africa), | Tibet, N. Asia, Japan), |
| <i>Micromeria biflora</i> Benth., | <i>Youngia japonica</i> (L.) DC., var. <i>genuina</i> |
| <i>M. capitellata</i> Benth., | (Hochr) Babcock and Stebbins |
| <i>Pittosporum floribundum</i> W. & A., | (China, Afghanistan, Japan, Malaya, |
| | Mauritius). |

Arisaema tortuosum Schott., occurs in Provinces 1, 2, 3, 8, 9, 20, 21.

Species in Provinces 1, 2, 3, 8, 9, 10, 18-21 : *Plectranthus coesta* Buch. Ham., (Burma, Afghanistan).

Species in Provinces 1, 3, 8-11, 18-21 : *Launaea acaulis* (Hook f) Babcock.

Species in 1, 2, 8-11, 18-21 : *Habenaria commelinifolia* Linn.

Species in 1, 8-11, 18-21 : *Limnophila hypericifolia* Benth.

Species in 1, 2, 3, 18-21 : *Rubus gardnerianus* O. Ktz. (Burma, Malay Archipelago); *R. niveus* Thunb. (Burma, Java).

Species in Provinces 1, 3, 18-21 : *Galium asperifolium* Wall. (Burma, Ava, Martaban); *Eulalia quadrivalvis* O. Ktz., var. *wightii* Hook f., *Fragaria indica* Andr. (Afghanistan, China, Japan, Korea, Penang, Malaya).

Species in Provinces 1, 2, 8, 9, 18-21 : *Dumasia villosa* DC. (Java, Madagascar, Natal); *Hydrocotyle javanica* Thunb. (Pegu, Tenasserim, Malaya, Philippines, Madagascar).

Species in 1, 8, 9, 18-21 :

- Blumea hieracifolia* DC. (Java),
Calamintha umbrosa Benth. (Afghanistan, Caucasia, China, Japan, Java),
Conyza viscidula Wall. (Burma, Java, New Caledonia, Philippines, Australia, Africa),
Desmodium parviflorum DC. (Burma, China, Japan, Malaya),
Lipocarpa argentea R. Br. (tropics and subtropics of the Old World),
Microstegium ciliatum A. Camus.,
Phoebe lanceolata Nees.,
Polygonum minus Huds. (Europe, tropical and temperate Asia),
Potentilla kleiniana W. & A.

Species in 1, 8, 9, 18 : *Conyza aegyptiaca* Willd. (Africa, China, Japan, Australia).

Pattern D shows the following groups:—

Species in Provinces 1, 2, 3, 20-21 :

- Impatiens chinensis* Linn.,
Luisia teretifolia Lindl. (Malaya to New Caledonia),
Neolitsea zeylanica Merr. (Burma, Malaya, Penang),
Olea glandulifera Wall.,

Paramignya monophylla Linn.,
Phoebe paniculata Nees.,
Rauwolfia densiflora Benth. & Hook f.,
Tylophora macrantha Hook f.

Species in Provinces 1, 2, 8, 9, 18: *Allophylus rheedii* Radlk.,
Plectranthus incanus Link.

Species in Provinces 1, 3, 18: *Carex baccans* Nees.,
Clausena heptaphylla W. & A.,
Morinda umbellata Linn.

Species in Provinces 1, 8, 9, 18: *Macaranga indica* Wt.,
Polygonum pedunculare Wall.

Pattern *E* consists of the following groups:—

Species in Provinces 1, 18-21 are:

<i>Acalypha brachystachya</i> Hornem.,	<i>Mahonia leschenaultii</i> Takeda.,
<i>Agrostis pilosula</i> Trin.,	<i>Microstegium nudum</i> A. Cam.,
<i>Brachiaria semiundulata</i> Stapf.,	<i>Microstylis wallichii</i> Lindl.,
<i>Campanula ramulosa</i> Wall.,	<i>Parochaetus communis</i> Hamilt.,
<i>C. wightii</i> Gamble.,	<i>Peperomia heyneana</i> Miq.,
<i>Chambainia cuspidata</i> Wt.,	<i>Picris hieracioides</i> Linn.,
<i>Cnicus wallichii</i> Hook f.,	<i>Piper brachystachya</i> Lindl.,
<i>Cotoneaster burxifolia</i> Wall.,	<i>Pratia begonifolia</i> Lindl.,
<i>Elatostemma sessilis</i> Forsst.,	<i>Rhamnus virgatus</i> Roxb.,
<i>Epipactis consimilis</i> Wall.,	<i>Senecio intermedius</i> Wt.,
<i>Geranium nepalense</i> Sweet.,	<i>S. wightiana</i> DC.,
<i>Gnaphalium hypoleucum</i> DC.,	<i>Spiranthes sinensis</i> Ames.,
<i>Hypericum hookerianum</i> W. & A.,	<i>Thalictrum saniculaeforme</i> DC.,
<i>H. wightianum</i> Wall.,	<i>Torenia vagans</i> Roxb.,
<i>Jasminum bignoniaceum</i> Wall.,	<i>Vandellia nummularifolia</i> Lindl.,
<i>Laportea terminalis</i> Wt.,	<i>Viburnum coriaceum</i> Bl.,
<i>Luzula campestris</i> DC.,	<i>V. erubescens</i> Wall.

Anotis wightiana Benth. & Hook f. (Ava, Cochinchina, Malaya),
Brunella vulgaris Linn. (temperate zones of northern hemisphere, Australia),
Gentiana pedicellata Wall. (China, Burma, Java),
Gymnostemma pedata Bl. (Japan, Malaya),
Polygala sibirica Linn. (Siberia, China, Japan),
Rumex nepalensis Spreng. (Java, westward to Asia and South Africa),
Sagina procumbens Linn. (Tibet, north and south temperate zones),
Sanicula europaea Linn. (Malaya, Europe, Asia, Africa).

Species in Provinces 1, 20-21 include:

<i>Agrostis stolonifera</i> Linn.,	<i>C. longicruris</i> Nees.,
<i>Anemone rivularis</i> Ham.,	<i>C. longipes</i> D. Don., var. <i>dissitiflora</i> Cl.,
<i>Arthraxon rudis</i> Hochst.,	<i>C. maculata</i> Boott.,
<i>Arundina graminifolia</i> Hochr.,	<i>Desmodium scalpe</i> DC.,
<i>Arundinaria fuscata</i> Nees.,	<i>Dianella ensifolia</i> Red.,
<i>Avenastrum asperum</i> Fischer.,	<i>Elatostemma acuminata</i> Brongn.,
<i>Calamintha masuca</i> Lindl.,	<i>Epipogon nutans</i> Reichb. f.,
<i>Campanula fulgens</i> Wall.,	<i>Eriocaulon melaleucum</i> Mart.,
<i>Carex filicina</i> Nees.,	<i>Fragaria neelgherrensis</i> Schlecht.,
<i>C. jackianus</i> Boott.,	<i>Gardneria ovata</i> Lindl.,

Gentiana quadrifaria Bl.,
Glochidion fagifolium Hook f.,
Justicia latespicata Gamb.,
Leucas zeylanica R. Br.,
Liparis pusilla Ridl.,
L. viridiflora Lindl.,
Lonicera ligustrum Wall.,
Lysimachia ovata Buch Ham.,
Mastixia arborea Clarke.,
Parnassia mysorensis Heyne.,
P. wightiana Wall.,
Pentapanax leschenaultii Seem.,
Phoebe wightii Meissn.,

Pilea wightii Wedd.,
Potentilla leschenaultiana Ser.,
Procris wightiana Wall.,
Rapanea wightiana Mez.,
Sageretia hamosa Brongn.,
Siccrpus fluitans Lindl.,
Scutellaria rivularis Benth.,
Senecio walkeri Arn.,
Smithia blanda Wall.,
Ternstroemia japonica Thunb.,
Tropidia angulosa Bl.,
Viola distans Wall.,
Youngia fuscipappa Thw.

Species in Provinces 1, 2, 18, 20-21 :

Eulophia herbacea Lindl.,
Parietaria debilis Forst. (many temperate and tropical regions extending to Australia and Fiji),
Polygala persicariaefolia DC. (tropical Africa, Australia).

Species in Provinces 1, 2, 18 :

Arenaria neelgherrensis W. & A.,
Cheirostylis flabellata Wt.,
Digitaria ternata Walp.,
Meliosma arnottiana Walp.

Species in 1, 2, 19-21 : *Rubus fairholmianus* Gardn. (Borneo, Malaya).

Species in 1, 18, 20-21 : *Korthalsella japonica* Edgew.,
Scutellaria paniculata Edgew.

Species in Provinces 1, 19-21 :

Carex hebecarpa C. A. Mey., var. *ligulata* Kuenth. (China, Japan),
Gaultheria fragrantissima Wall. (Burma, Malaya),
Polygala arillata Buch Ham. (South China, Ava, Malayan Archipelago),
Stellaria saxatilis Ham. (Siberia, Japan, Java).

The following groups of species form Pattern F.

Species in 1, 2, 3, 10-11 : *Eriocaulon longicuspis* Hook f., var. *polycephala* Fyson.

Species in 1, 3, 10-11, 18-21 : *Ophiopogon intermedium* D. Don., *Sarcococca trinervia* Wt. (Afghanistan, Sumatra).

Species in 1, 2, 3, 14, 18-21 : *Lecanthus wightii* Wedd. (Java, Africa).

Species in 1, 2, 3, 8, 9, 18-21 : *Artemisia vulgaris* Linn. (Siam, Java, Africa, Australia).

Species in 1, 2, 14, 18-21 : *Vernonia conyzoides* Wt. (tropical Asia, Africa, Australia).

Species in 1, 2, 14, 18 : *Linum mysorense* Heyne.

Species in 1, 18 : *Ranunculus muricatus* Lindl. (Europe, West Asia, temperate North America).

Species in 1, 18, 19 : *Juncus glaucus* Ehrh. (Europe, N. Asia, N. Africa).

Species in 1, 14, 18, 20-21: *Carex breviculmis* R. Br. (China, Japan, Australia, New Zealand).

The undermentioned groups of species constitute Pattern G.

Species in Provinces 3, 20-21:

Disporum calcaratum D. Don.,
Emilia bambusifolia Lindl.,
E. scabra Don.,
Gymnosporia acuminata Hook f.,
Pericampylus incanus Miers.,
Strobilanthes theaeifolia D. Don.,
Uncaria sessilifructus Roxb.

Species in Provinces 3, 8, 21:

Anotis calycina Wall.,
Cinnamomum caudatum Nees.
Calamus latifolia Roxb., occurs in Provinces 3, 8, 9, 11, 18.

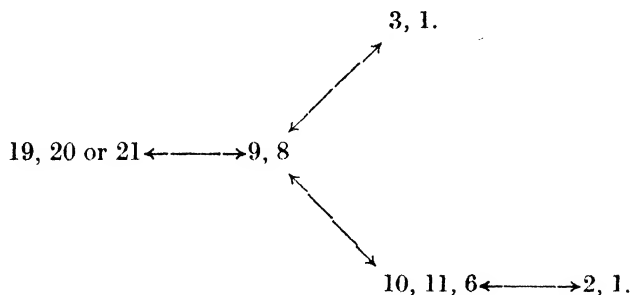
Species in Provinces 3, 8, 9, 18-21:

Dendrobium bicameratum Lindl.,
Ehretia acuminata R. Br. (China, Japan, Burma, Malaya, N. Australia),
Senecio nudicaulis Buch Ham.

These distribution patterns indicate certain migratory tracts. It will be noticed that in most of the patterns there is a prevailing south to north-east trend, and in many of these the routes could have been:—

1 \longleftrightarrow 3 and/or 8, 9 \longleftrightarrow 20-21.

The distribution pattern C and its variants indicate the possibility of this route. The possibilities of migration are:—



It has already been remarked while enumerating pattern B that there could have been a possible intermigration of species between the mountains of the east and the west, or in the alternative these could have been derived from some common source. The common source could have been the provinces Bihar and Orissa.

Thus it appears that Provinces 8 and 9 have played an important rôle in the migration of plants from South-west to North-east India or vice versa. This fact confirms the belief of earlier workers like Hooker and Thomson (1855), and Fyson (1915-21, 1932). Hooker and Thomson (*l.c.*, p. 126) say that the ravines and the shady parts near the undulating slopes of the Nilgiris are occupied by thickets and small bushes like those of Ceylon, but probably composed of greater number of species all of which are equally characteristic of similar situations in the Khasias.

Fyson (1932) says that some 17% of the species occur on the Khasia hills, 1,500 miles away, and about 12% on the temperate parts of the Himalayas, but practically none at all in the intervening country, even along the Western Ghats.

These two authors have thus shown the similarity between the plants of South Indian hills and those of Himalayas; and the same has been hinted at by Chatterjee (1940). The sweeping statement made by Fyson that there are no common plants in the intervening countries does not receive support from the present study. In fact the recurrence of Provinces 3, or 3, 8 and 9 in most of the distribution patterns appears to be directly opposed to Fyson's views.

The present study has thus confirmed the similarities between the high altitude floras of the South Indian hills and the Himalayas, and has also suggested probable routes of migration between the two areas. The question still remains as to direction in which migration has taken place.

It should be remembered that the area with which we are concerned is one of the oldest known land masses of the earth, and as such it is reasonable to expect that it has been covered by some sort of vegetation from the early times. This assumption can naturally be extended to the belief that there was some vegetation in this area even before the advent of the Himalayas. This belief happens to be true as evidenced by the facts of the area forming part of the old Gondwana continent. As to the exact nature of the old vegetation there are no means of finding out at present, particularly so in the absence of fossil data.

The work of several persons indicates that the flowering plants originated on the southern land mass. Among these, we can notice the opinions of two eminent phytogeographers. Hill (1926, p. 1480) in his masterly essay on 'Antarctica and problems of plant distribution' remarks that '*Vernonia*, on the present day evidence appears to belong to the south, and from a primitive species, which probably reached South Africa, South America and India—from Antarctica, that modern species have originated'.

Camp (1946) while dealing with the distribution patterns in modern plants and the problems of their ancient dispersals adduces evidence which makes it abundantly clear that the southern land masses appear to be the possible areas of origin in so many groups of land plants. It seems likely that angiosperms as a group arose on the southern land mass contemporaneous with the palaeozoic of the northern (holartic) land mass and that the divergence of the familial groups had been accomplished on this southern mass certainly by the mid-Mesozoic. Further, Camp is of the opinion that a great bulk of the angiosperm families and many of their present day genera appear to have evolved by the Cretaceous, and that this angiosperm group began moving northwards, so that by the Eocene at least sub-tropical genera became abundant on the lower parts of the northern land mass. These observations of Camp are directly opposed to the view generally held that the angiosperms, as all other plants, arose in the north and that there has been a southward migration. A good account of the latter view is given by Just (1947).

Whatever the case, or the merits and demerits of the two views, for the sake of the present study it can be assumed that migration has occurred both ways, for, as far as migrations of plants are concerned, there can be no one-way traffic. Among those plants that have come from a northern source can be mentioned species of *Hypericum*, *Parnassia*, *Thalictrum*, *Gordonia*, *Clematis*, *Rubus* and *Lonicera*; while among plants that have originated in the south can be mentioned *Procris*, *Heynea*, *Allophylus*, *Pygeum*, *Schefflera*, *Mastixia*, *Maesa*, *Isonandra*, *Symplocos*, *Cryptocarya*, *Cinnamomum*, *Litsea*, *Neolitsea*, *Phoebe*, *Daphniphyllum* (Malaysian), *Lobelia* and *Conyza* (African) as established by Razi (in press).

In this connection, the views of Mooney (1942) on the occurrence of South Indian plants on the Bailadila Range in Bastar State of Orissa are interesting. He says that it does not call for great powers of imagination to visualize how species having their origin in the Nilgiris, Pulneys and other hills of Mysore, Travancore

and Southern India may have travelled along the line of the Eastern Ghats until they reached their northern extremity in Kasipur Plateau of Kalahandi State, and the Agency tracts of Ganjam and Vizagapatam districts with their humid coastal climate and many hills exceeding 4,000' in altitude. The distance from the southern plateau of Kalahandi to Bailadila in a straight line is not more than 120 miles; but Tulsi Dangi, itself 3,914' situated about 150 miles east of Bailadila and other intermediate hill ranges of lesser calibre shorten the gap. Mooney, thus presents clear evidence of the south to north migration in the occurrence of 32 species of South Indian plants in the Bailadila range. On the other hand, he found it difficult to explain the occurrence of the 36 north-east Indian plants in Bailadila. For explaining the occurrence of these north-east Indian elements, Hora (1949) has recourse to his Satpura hypothesis when he says that though present-day topographic and climatic conditions are not favourable for the plants and animals of the Assam and the Eastern Himalayas to migrate to Chota Nagpur over the Rajmahal hills, the Satpura hypothesis postulates the existence of such favourable conditions over the intervening area in the Pliocene and Pleistocene times. Thus, Mooney presents evidence to show a northward migration from South India, while Hora postulates a southward migration from the north-east. As far as the present study is concerned, both views can be taken to be correct.

An example of a genus which is northern, but on the strength of present-day evidence appears to have produced at least a Section in the south, is *Youngia*. Babcock and Stebbins (1937) say that the distribution of the genus taken as a whole is entirely consistent with the conception that it is a natural group which had its origin in South-east Asia and that evolution has been accompanied by an extension of the geographic range to its natural limits on the south. The distribution of most species of *Youngia* overlaps in the area comprising Eastern Himalayas, Tibet, Assam, South-west China; and as such this area can be taken to be the place of origin for the genus. There are three species in South India:—

1. *Youngia fuscipappa* Thw., Western Ghats in Avalanche and Sispara in Nilgiris; Ceylon in highlands.
2. *Y. nilgiriensis* Babcock—Sispara in Nilgiris at about 2,060 m.
3. *Y. japonica* (L.) DC., subsp. *genuina* (Hochr) Babcock and Stebbins—Japan and Korea to West China, Malay Peninsula to North-west India; Philippines; North Circars in the hills of Ganjam, Western Ghats in the Nilgiris, Pulneys and hills of Travancore and Tinnevelley. Babcock and Stebbins (1937) say that this species is introduced widely in the tropics.

The first two belong to Section 5. Mesomeris of Babcock and Stebbins (1937), while the third belongs to Section 6. Euyoungia. *Y. nilgiriensis* is intermediate between *Y. gracilis* and *Y. cineripappa*, both of which belong to Section Mesomeris. Babcock (1939) who described the species *Y. nilgiriensis*, is of the opinion that several factors characterize it as a somewhat more primitive species than the others in Mesomeris. He suggests that its occurrence in an isolated highland may be due to its relict nature.

The diagnostic characters of the species *Y. nilgiriensis* indicate that it may probably represent an ancestral type from which the Section Mesomeris arose. If *Y. nilgiriensis* is the progenitor of this subsection of Mesomeris, it becomes necessary to believe that the progenitor ranged from the centre of the genus in the Sino-Himalayas right down to South India, and that changing climatic conditions have wiped out all its traces in the intervening areas. As to whether it originated in the north or the south it is not possible to decide on the strength of present data. The possibility of a southern origin of this subsection is a fascinating hypothesis which remains difficult of proof, but within the bounds of possibility.

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SUMMARY

In the present study an analysis is made of the distribution of 370 plants occurring on hilltops in Madras State and Mysore, out of a total of 1,224 species so far reported.

The analysis is based on a division of India into 22 botanical provinces, and the occurrence of species in one or more of these.

Distribution patterns are adduced for groups of species, and these are indicative of routes of migration.

In the majority of the distribution patterns there is a distinct south to north-east trend.

The analysis reveals that the provinces Bihar and Orissa have played an important rôle in this north-south migration.

Possible routes of migration are indicated on the assumption that migration has occurred both ways. Examples are given.

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OBSERVATIONS ON THE IONIC COMPOSITION OF BLUE-GREEN ALGAE GROWING IN SALINE LAGOONS

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INTRODUCTION

The capacity of certain Myxophyceae to withstand marked alterations in salinity of the medium as well as the presence of some of them in salt marshes have been reported by various authors, viz., Cavara (1902) (*vide* Brooks and Brooks, 1947), Ercevoic (1930), Prat (1925) (Fritsch, 1945), and Carter (1933). The abundant occurrence of some of the species of blue-green algae in solar salt marshes has been noticed by Hansgirg (1887) and Hof and Frey (1933) (*vide* Fritsch, 1945), chief among these being *Microcoleus chthonoplastes*, a species often associated with *Lyngbya aestuarii*. Hof and Frey have distinguished the algae as halotolerant and halophilic species, the latter term denoting those which can grow and multiply in solutions more concentrated than three molar NaCl, applicable to a few Chroococcales (esp. *Aphanocapsa littoralis*) and forms like *Spirulina subsalsa* and *Phormidium tenue*. The vertical expansion and contraction of filaments of blue-green algae esp. *Oscillatoria jenensis* and their relationship to osmo-regulation in the algae have been studied in detail by Schmid (1923). In their monograph Brooks and Brooks (1947) have discussed the degree of permeability in blue-green algae which can withstand relatively concentrated brines whose osmotic pressures are about 150 atmospheres. They observe that these cells are permeable to water in both directions in response to the osmotic forces. But no quantitative data are given to substantiate the statement and to explain the various physiological changes taking place in the algal cells under varying salinity conditions.

Nakamura (1938) discusses the process of carbon assimilation of lower algae in the presence of hydrogen sulphide. Instead of water, H₂S is the source of hydrogen for the reduction of CO₂, the evolution of oxygen in this case being replaced by the deposition of sulphur within the cells.

As the salinity rises in the waters of saline lagoons, corresponding changes in the other constituents naturally follow, but in degree and rate of change they need not necessarily be proportional to the changes in the chloride content. Howes (1939) observes that the relative proportion of salts in estuaries and salt marshes need not be the same as in sea water. He has collected data from a saline lagoon

in East Essex and shows that there is a significant rise in the sulphate content of the water. Also there is a slow decrease in the ratios K/Na, Ca/Na and Mg/Na, whereas the ratio $\frac{\text{Ca} + \text{Mg}}{\text{K} + \text{Na}}$ remains almost the same as that of normal sea water.

Such differences in the relative proportions of salts between lagoon waters and normal sea water are explained as being due to the differential precipitation during evaporation (Beadle, 1943).

The differences in the ionic proportions will be accentuated in inland salt water lagoons, and the survival of plant and animal species under these conditions will depend upon special adaptations, the fauna and flora being subjected to the wide fluctuations in the ionic composition of the waters.

Apart from the major constituents mentioned above several other minor elements also may contribute to the healthy growth of algae in waters. In recent years a few attempts have been made to study the growth requirements of a number of blue-green algae. Gerloff, Fitzgerald and Folke Skoog (1950*a, b*) describe procedures employed in the continuous culture of about 22 species of blue-green algae including species of *Microcystis*, *Aphanizomenon* and *Lyngbya* and some others which have not previously been maintained in cultures. These studies bring to light the importance of several minor elements especially iron in the metabolism and growth of these algae. Excellent summaries of the nutritional requirements of other forms, especially among the green algae, have been given by Mainx (1929), Molisch (1895, 1896) (*vide* Stiles, W. 'Trace elements in plants and animals', 1948), etc., which bring to light the importance of a number of minor elements such as manganese, iron, copper, iodine, boron, etc., in the metabolism and distribution of algae. The manganese and iron requirements of *Chlorella* have been studied by Hopkins (1930, 1938) and Hopkins and Wann (1927) respectively, and of the diatom *Ditylum brightwelli*, by Harvey (1939). Roberg (1932) found that autotrophic chlorophyceae could not grow in the absence of iron and reports increased growth of two unicellular green algae, *Coccomyxa simplex* and *Chlorella vulgaris* as a result of small additions of salts of iron, zinc and copper to the normal nutrient solutions. Uspenski (1924 and 1925—*vide* Bold, 1942) has worked on iron as a factor in the distribution of algae in the Russian waters. As regards the lagoon waters there is every possibility of an almost perpetual shortage in some of the essential minor elements as supplies of sea water are cut off periodically, and even when the lagoons are in contact with the sea the entry of sea water is often insufficient for adequate irrigation and replenishment of nutrients.

EXPERIMENTAL

The present experiment is a study on some of the problems indicated above and is calculated to gather information on the following points:

- (1) Variation in the intensity of the growth of lagoon algae with rise in salinity and their limits of salinity tolerance,
- (2) The variation in the chemical composition of lagoon waters and the possible effect of the abnormal rise in the chloride content on the ratios between other ions,
- (3) Ionic changes taking place in the cell fluid of certain algal species as well as of the algae as a whole growing in the lagoons, and
- (4) Differences in the amounts of some of the minor elements present in the water and in the algae.

Two stations which represented two different conditions were selected from Palk Bay Lagoons at Mandapam Camp. The first station was selected from the major lagoon extending over several hundred acres. From October till the end of

March it had connection with the sea and was under tidal influence, while the second one was separated from the first even in the beginning of March, though it originally formed part of the main lagoon. Naturally the rate of concentration of salt was greater in station II than in station I from March.

Samples of bottom algae and water were separately collected from the above stations at definite intervals. The algal collections did not contain any other types of algae other than blue-greens though almost all the collections showed presence of the diatom *Nitzschia vitrea* (Krishna Pillai, 1954). The water samples were analysed for Na, K, Ca, Mg, Fe, Mn, Cu, I, B, Zn, N (both protein and non-protein nitrogen) and S contents. The cell fluids were extracted from the fresh algae by grinding them well with acid-washed sand and pressing out the fluid at high pressure. The fluid thus obtained was centrifuged and used for analysis.

METHOD OF ANALYSIS

The methods employed for the analysis of Na, K, Ca, Mg, Cu and Cl contents of the water and of the algae and their cell fluid were those employed by Robertson and Webb (1939) for biological materials. B.D.H. micro analytical reagents were used in the estimations. In the case of the estimation of potassium a slight modification has been made; instead of dissolving the precipitate of potassium cobaltinitrite in ceric sulphate, the Kramer and Tisdall's volumetric method has been used.

The total iron in the algae was estimated by first igniting accurately weighed quantities of air-dried samples, digesting with excess of concentrated HCl and applying the potassium thiocyanate method of colour comparison, the final comparison being made in Hilger Spekker Photo-electric Absorptiometer (A.O.A.C., 1945, p. 157).

Iodine was determined by the method specially adapted for biological materials and used by Von Fellenberg (1924).

Sulphate in water was determined by the usual method of precipitation and weighing as BaSO_4 . Total sulphur in the algae was determined by the method employed by Aitken (1930).

Zinc was estimated by the method suggested by Hibbard (1934), while manganese in sea water and in the algae was determined by the periodate method given by Snell and Snell (1949).

Total as well as non-protein nitrogen were estimated by the micro-kjeldahl technique (Hawk, Oser and Summerson, 1947) absorbing the ammonia in 2% boric acid and finally titrating against standard N/70 sulphuric acid.

After each collection the total wet weight of the algae from a unit area of each station was estimated so that the variations in the algal production at these stations could be studied. The values are tabulated in Table I. An attempt was also made to study the variations in the amount of particular species growing under varying salinities, which, however, was not successful owing to the difficulty in isolating each species. A complete qualitative analysis of the algal samples was done and the component species with their relative amounts noted (Krishna Pillai, 1954). The results of the chemical analysis of the algae and water are given in Tables I and II respectively. In the case of water samples values are given only for the Fe, Mn and Cu contents, as the presence of other minor elements, viz., B, I and Zn could not be detected owing either to their complete absence or to insufficient sensitivity of the methods employed.

TABLE I
Trace element content of blue-green algae
(On oven-dry basis)

	Total wet weight of algae g./sq. metre	Dry weight %	Ash %	Protein-N %	Non-protein-N %	Fe	Mn	Cu (parts per million)	I	B	Zn
STATION I—											
(i) ..	930.0	18.3	55.4	0.60	0.52	73.0	1.2	12.5	48.6	Trace	5.0
(ii) ..	635.0	20.7	54.0	0.41	0.49	15.5	<0.1	7.3	41.0	Nil	<1.0
(iii) ..	520.0	46.2	60.3	0.23	0.40	30.8	<0.1	Trace	47.6	Nil	<1.0
STATION II—											
(i) ..	690.0	19.3	68.5	0.47	0.41	55.0	0.4	12.5	24.3	8.0	Nil
(ii) ..	380.0	23.6	70.9	0.21	0.40	2.0	<0.1	Trace	Nil	Nil	Nil
(iii) ..	250.0	49.5	71.0	0.19	0.39	7.8	<0.1	Nil	Nil	Nil	Nil

TABLE II
Chemical composition of water from the two stations

	pH	Na mg./litre	K mg./litre	Ca mg./litre	Mg mg./litre	Cl mg./litre	SO ₄ mg./litre	Fe μg./litre	Mn μg./litre	Cu μg./litre
STATION I—										
(i) ..	8.5	9,430	60	910	791	19,880	3,460	14	27	31
(ii) ..	8.2	26,430	90	1,360	2,740	47,860	4,020	Trace	..	10
(iii) ..	8.4	71,230	1,510	1,550	2,705	111,720	11,840	Nil	..	10
STATION II—										
(i) ..	8.4	8,800	40	1,090	1,660	17,320	3,430	8	Nil	10
(ii) ..	8.2	27,190	80	1,280	5,600	60,670	3,790	Trace	Trace	Nil
(iii) ..	8.4	56,230	1,520	2,760	7,160	105,120	11,210	Trace	Trace	10

The ionic composition of the cell fluids and the external medium have been tabulated in Tables III and IV.

TABLE III

Ionic composition of water and algal cell fluid from Station I

(Expressed in mM concentrations)

	I		II		III	
	Water	Cell fluid	Water	Cell fluid	Water	Cell fluid
Na	410.0	123.0	1,149.2	130.8	3,097.0	Nil
K	1.5	11.0	2.3	26.0	38.8	36.4
Ca	22.8	45.3	34.2	64.5	38.8	83.7
Mg	32.5	50.3	112.7	68.7	111.0	90.0
Cl	580.7	37.4	1,350.0	45.1	3,151.5	37.0
SO ₄	35.0	98.2	42.0	180.3	123.3	244.8

TABLE IV

Ionic composition of water and cell fluid from Station II

(Expressed in mM concentrations)

	I		II		III	
	Water	Cell fluid	Water	Cell fluid	Water	Cell fluid
Na	382.0	112.6	1,182.2	Nil	2,444.8	Nil
K	1.1	8.2	2.1	29.0	39.0	33.6
Ca	27.3	24.6	31.9	65.7	69.0	80.0
Mg	67.2	23.2	230.0	65.0	294.0	79.0
Cl	488.7	16.6	1,711.0	40.1	2,965.0	27.8
SO ₄	35.7	156.4	39.4	218.4	116.8	274.2

The changes in the ratios between Na and K, Ca and Mg, and (Ca+Mg) and (Na+K) in the waters in the two stations are presented in Table V.

TABLE V

Ratios between cations in water

Observations	STATION I			STATION II		
	K/Na	Ca/Mg	$\frac{Ca+Mg}{Na+K}$	K/Na	Ca/Mg	$\frac{Ca+Mg}{Na+K}$
1	0.0065	1.15	0.178	0.0046	0.67	0.300
2	0.0034	0.50	0.150	0.0029	1.60	0.250
3	0.0210	0.55	0.57	0.0190	1.98	0.173

DISCUSSION OF RESULTS

The production of algae shows a definite decrease from the first collection to the third in both the stations. It is noteworthy that none of the stations contains any varieties of algae other than the blue-greens. At station I during the first collection when it had connection with the sea oscillatorians constitute the major part of the algal population while *Phormidium*, *Aphanothece* and *Spirulina* are present only in very small quantities. In station II which is isolated and detached from the major lagoon, *P. tenue* constitutes the bulk of the algae throughout the period of the experiments. This suggests that the conditions offered by this pond is not favourable to any of the above blue-greens except *P. tenue*. But even in this case in the second and third collections the bulk of the algae was decomposing with the liberation of hydrogen sulphide.

In station I also the percentage of *P. tenue* in the collection increased with the rise in chloride content of the water and all the other algae originally present in the collections disappeared.

The total nitrogen content of the algae shows a definite decrease. This decrease is more evident in the protein nitrogen rather than in the non-protein (Table I) indicating probably that the protein is being disintegrated. The total nitrogen and the ash content of the algae are comparatively lower than the values reported by Schuster (1949) for blue-green algae. Among the minor elements only Fe, Mn, Cu, B, Zn and I have been studied.

Table II gives the variations in the chemical composition of the water including those in the minor elements. The samples do not contain zinc, iodine and boron. Their absence cannot be ascribed to the lack of sensitivity of the methods adopted, which can detect these elements even in low concentrations (10 μ g. in a litre). Iron, manganese and copper found in the water samples are very low when compared to the values reported for normal sea water by Black and Mitchell (1952). Thus it is clear that the water in the lagoons is deficient in the above minor elements and to this extent cannot provide the true and natural conditions in sea water.

It may be seen from Table I that as the salinity of the outside water increases the water content of the algae slowly goes down (represented by a rise in dry matter), which would naturally lead to a concentration of the ions in their cell fluids. But this increase in the ionic concentration is most marked, among cations, only with K, Ca and Mg. As these ions increase, the concentration of Na ion does not show any significant change until the chloride concentration of the external medium rises to 1,711.0 mM, when all the Na from the cell fluid is given out (Table IV). It may be seen from Tables III and IV that the variation in the concentration of Na ion in the cell fluid of algae from the two stations is only between 112.6 mM and 130.8 mM, while the concentration of Na ion in water varies from 383 mM to 1,182 mM.

Fig. 1 drawn from the actual weights of the various ions in 1 c.c. each of the solutions shows that when the chloride content of the outside water is 560.7 mM in station I, Na constitutes 45% of the cation content of the cell fluid. With rise in salinity to 1,350 mM chloride the percentage of Na ion falls to 36 in station I. But in station II when the chloride content reaches 1,711 mM the percentage of Na in cell fluid falls to zero the cation composition of the last being Na = nil; K = 20%; Ca = 48% and Mg = 22%. This means that between these chloride concentrations (i.e. between 1,350 mM and 1,711 mM) complete elimination of Na must have taken place.

Subsequent observations on the cell fluid of the algae from both stations show that the percentage of cation remains the same even when the salinity of the outside water goes up to a chloride content of 3,151.5 mM. This may indicate that beyond a certain salinity at which complete elimination of Na from the cell fluids takes place, no cation inter-change occurs between the external medium and the cell

fluids of the algae or, in other words, that some sort of plasmolysis of the algal cells takes place when the chloride content of the external medium reaches 1,711 mM and that the cell fluids remain passive afterwards. The high values for the cations in the cell fluids during the third set of observations can only be due to loss of water from the cells during plasmolysis. The percentage composition of the cations remains the same after plasmolysis (Fig. 1) even though the composition of the external medium alters considerably indicating probably that no selective absorption of ions has taken place after plasmolysis.

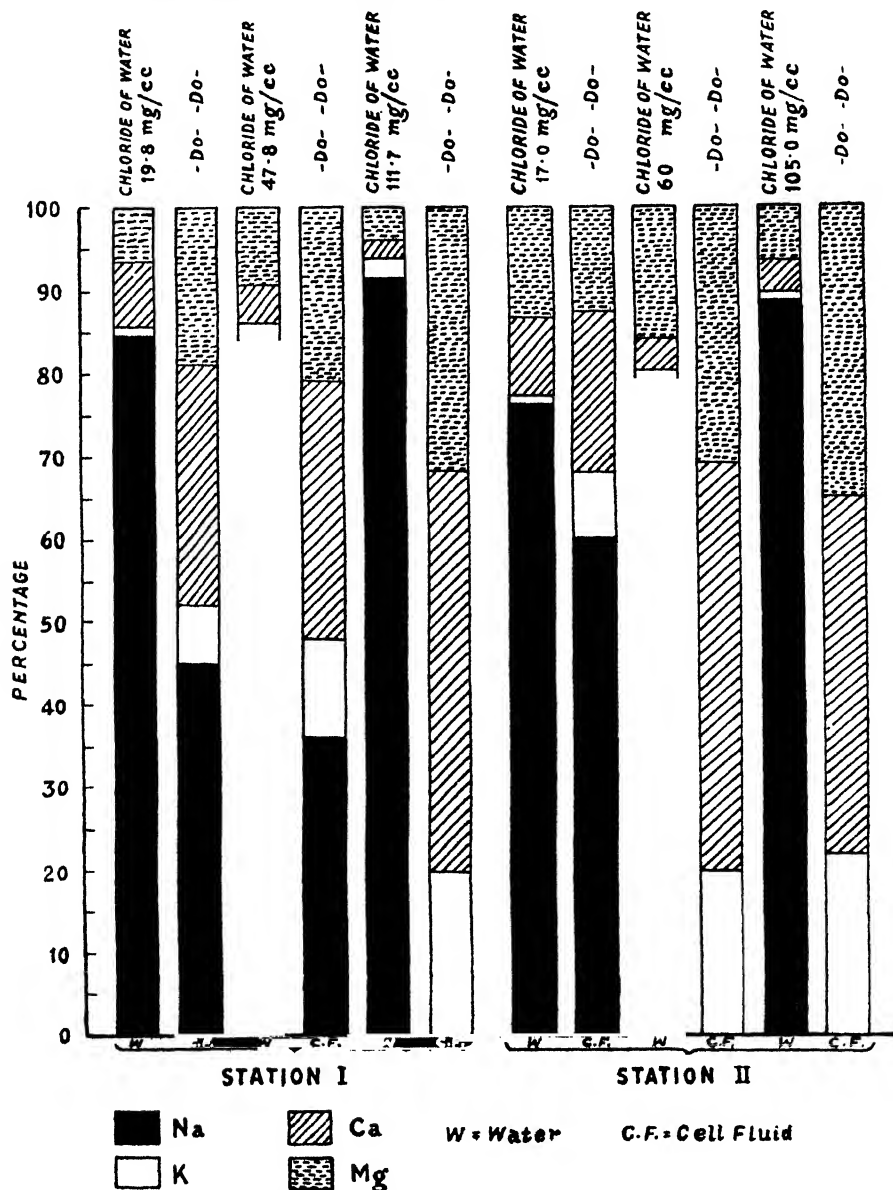
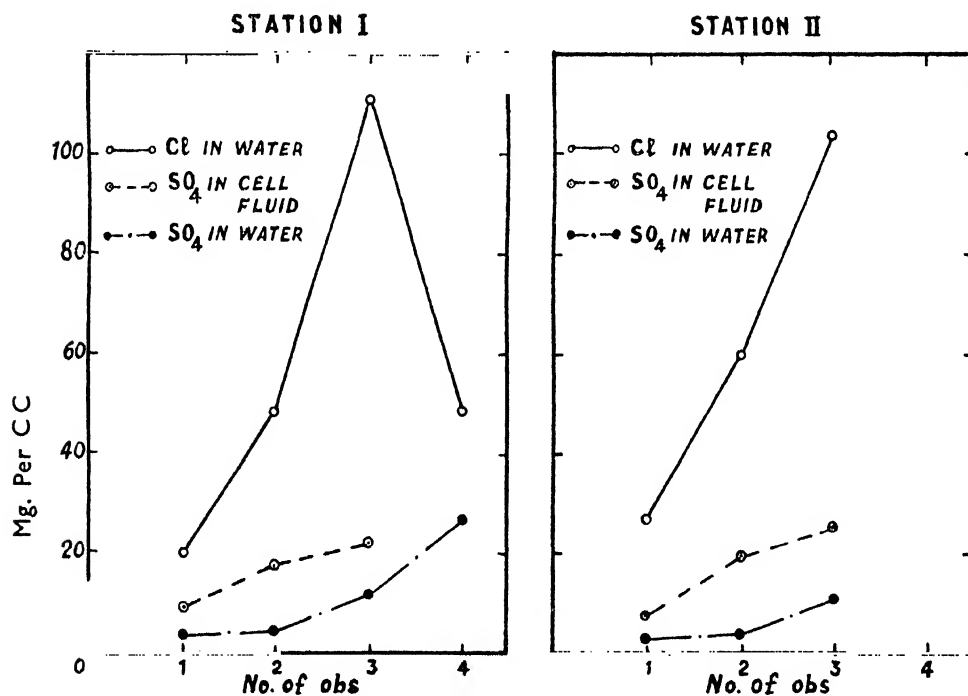


FIG. 1. The ratios between Na, K, Ca and Mg expressed in percentage assuming the total of the four ions to be 100.

In the case of anion exchange also certain peculiarities are noticed. Even though the changes in the chloride content of the external medium are very great, no such high values are noticed in the chloride content of the cell fluid. The changes in the inside chloride are so negligible that the ratio between the external and internal chlorides also increases and is almost directly proportional to the chloride content of the external medium. This fact is highly significant and proves beyond doubt that the blue-green algae, especially *P. tenue*, can withstand wide variations in the salinity of the external medium without allowing free passage of Cl ions into the cell fluids.

The cell fluid of the algae not being affected by the high salinity of the outer medium, the reason for plasmolysis and subsequent destruction cannot be ascribed to the chloride content of the water. Some other factor might be responsible for plasmolysing the algal cells. As mentioned above there is a significant rise in the SO_4 content of the cell fluid as well as of the external medium. Figs. 2 and 3 show



FIGS. 2 and 3. Variation in the Cl and SO_4 of water and cell fluid of the algae at the two stations.

that there is a gradual increase in the SO_4 content of the cell fluids of the algae until the chloride content of the external water reaches the vicinity of 6.0%. Thereafter there is practically no increase in the SO_4 content as the curve tends to droop down in both cases. On the other hand, the changes in the SO_4 content of the external medium is very low at the beginning until the chloride content reaches 6.0%, after which there is a steady increase. At some stage beyond a chloride content of 11.2%, sodium chloride crystallises out from the water and the resultant chloride content of the water falls considerably, but the SO_4 continues to increase. So the behaviour of SO_4 ion in the cell fluid is just the reverse of that of Cl ion, and

TABLE VI

	STATION I						STATION II					
	Observation 1			2			3			1		
	Cell fluid mg./c.c.	Algae corresponding to 1 c.c. cell fluid in mg.	Cell fluid mg./c.c.	Algae corresponding to 1 c.c. cell fluid in mg.	Cell fluid mg./c.c.	Algae corresponding to 1 c.c. cell fluid in mg.	Cell fluid mg./c.c.	Algae corresponding to 1 c.c. cell fluid in mg.	Cell fluid mg./c.c.	Algae corresponding to 1 c.c. cell fluid in mg.	Cell fluid mg./c.c.	Algae corresponding to 1 c.c. cell fluid in mg.
Na ..	2.8	2.8	3.0	2.9	Nil	Nil	2.6	2.5	Nil	2.5	2.6	Nil
K ..	0.4	0.4	1.0	1.1	1.4	1.4	0.3	0.3	0.3	0.3	0.3	1.3
Ca ..	1.8	2.1	1.6	2.7	3.4	3.4	0.8	1.1	2.6	3.8	1.2	3.2
Mg ..	1.2	1.7	1.7	2.2	2.2	2.2	0.6	0.9	1.6	1.9	1.6	1.9
Cl ..	1.3	1.1	1.4	1.1	1.3	1.1	0.6	0.6	1.6	1.5	0.9	0.9
SO ₄ ..	9.4	..	17.3	..	23.7	..	15.0	..	21.0	..	26.3	..
Total S as SO ₄	13.1	..	23.1	..	31.3	..	18.3	..	26.3	..	32.9

the cell walls do not resist the entrance of the SO_4 ions. It may be that the high concentration of the SO_4 ion in the cell fluid that brings about plasmolysis of the cells. The exact significance and the actual rôle of the SO_4 ions in the metabolism of the blue-green algae remains to be understood. However, it may be seen from Table VI that most of the SO_4 ions that get into the cell fluid do not actually go into effective combination with the cell wall, but remain in the form of ionic sulphate in the fluid itself. The changes in the difference between the ionic sulphate in unit volume of cell fluid and the total sulphur (expressed as SO_4) in the algae corresponding to that volume of cell fluid is very small when compared with the rise in the ionic sulphate in the cell fluid (Table VI). The continuous evolution of hydrogen sulphide from the two stations during high salinities may be due to the bacterial decomposition of the dying algae which contain a high percentage of free SO_4 ions. Part of the SO_4 released by the dying algae remains unreacted by sulphur reducing bacteria which probably accounts for the high SO_4 content of the water after plasmolysis of the algae. It may be noted that the analysis of the cell fluids and total algae from the third collections have been conducted on a mixture of fresh and decaying algae, as it was found extremely difficult to separate the two from the samples which were in the form of a scum.

The ratios between the cations in the water of the two stations show wide variations (Table V). In station I the ratio between the actual weights of potassium and sodium (K/Na) at first decreases from 0.0065 to 0.0034 corresponding to an increase in the chloride content from 19.88 mg./c.c. to 47.87 mg./c.c. Thereafter the ratio increases and the value is 0.021 when the chloride content is 111.72 mg./c.c. The fact that the ratio of K/Na in normal sea water is much higher than 0.0065 shows that lagoon water is deficient in potassium or that all the potassium in the water is being absorbed by and concentrated in the algae. This latter explanation finds support in the increase of the potassium concentration of the cell sap corresponding to a decrease in the K/Na ratio in the water until plasmolysis takes place. After plasmolysis the K from the algae is liberated to the surroundings and consequently the value of the ratio K/Na increases.

A similar change can be noticed in the case of water in the second station also. The ratio decreases till the chloride content reaches 60 mg./c.c. and then increases. The ratio between Ca and Mg also fluctuates within varying limits. As in the case of K/Na, Ca/Mg first decreases in both the stations and then increases showing that there is selective absorption of Ca by the algae before plasmolysis takes place. Afterwards the Ca content of the water increases, probably due to decay of the algae and release of Ca into the water, and the ratio Ca/Mg again increases. The ratio $\frac{\text{Ca} + \text{Mg}}{\text{Na} + \text{K}}$ shows a general decrease.

SUMMARY

The growth of blue-green algae in the saline lagoons near Mandapam under varying salinity conditions has been studied. Algal growth decreases with rise in salinity in the lagoons and beyond a chloride concentration of 1,700 mM the algae begin to die and disintegrate. Many of the species, viz., *Oscillatoria*, *Spirulina* and *Aphanothece* seem to disappear when the chloride content of the surrounding water rises to the vicinity of 1,700 mM. The only blue-green alga that survives this extreme condition appears to be *Phormidium tenue*.

The ionic changes taking place in the cell fluids of the algae collected from two stations have been discussed in detail. The algae resist the very high concentrations of chloride in the surrounding water by not allowing free passage of the chloride ions into the cells. But instead there is great accumulation of SO_4 ions in the cell fluid. As plasmolysis takes place the algae slowly disintegrate and liberate SO_4 into the water.

K, Ca and Mg are accumulated by the algae, the absorption of Ca and K being especially significant. When plasmolysis takes place the Na ion is given out of the cell fluid.

Compared to normal sea water the lagoon waters are found to be deficient in Fe, Mn, B and Zn throughout the year; and this may possibly be one of the many reasons for the fall in the growth rate of algae.

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In view of the heavy cost of Printing and Publication, the Council desires to urge upon Authors of Papers the absolute necessity for *brevity of statement* and for *restricting to a minimum* the number of Plates, Text-figures and Tables.

MICRO-STRUCTURE OF COALS FROM THE HUTAR COALFIELD

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(Communicated by Dr. D. N. Wadia, F.N.I.)

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INTRODUCTION

The study of coal petrology is proving of considerable interest and importance in recent years but our knowledge of the microscopic constitution of Indian coals (Banerjee, 1932; Ganju, 1954) is yet not comprehensive. It is important that a systematic petrological examination of all the productive coal seams in the Indian coalfields is made with a view to ascertain the composition of these coals and also to know what effect the microscopic constituents may have produced on the chemical properties of these coals. At the same time it would be possible to elucidate the anatomy of plants which have largely contributed in the formation of these coals. The microscopic examination should also be of considerable help in studying the regional variation in the rank of coal as well as in the variation of rank with depth.

A beginning in the study of micro-fossil contents in the Indian coals with a view to ascertain their possible use in the correlation of coal seams has been made by Ghosh and Sen (1948, 1953); but extensive researches in this line have to be carried out before it is possible to estimate the importance of using microspores by themselves in the methods of correlation of Indian coals. The nature and distribution of megaspores in a coal seam may, however, prove of greater interest in the problem of correlation of coal seams.

The material which forms the subject of this paper was collected from the Hutar colliery during a visit to the Hutar and Daltonganj coalfields in December, 1952, by the author who was directing the field work of post-graduate students in that area at that time.

The Sone Valley Hutar colliery is situated in the Hutar coalfield in the Palamau district of Bihar at a distance of about two miles from the Barwadih station on the Dehri-on-Sone Gomoh line of the Eastern Railway.

GEOLOGY OF THE AREA

The Hutar coalfield is one of the three coalfields in the Palamau district, the other two being the coalfields of Aurunga and Daltonganj. This coalfield lies in the Valley of the Koel River and its eastern boundary is at a distance of about 12 miles to the west of the western limits of the Aurunga coalfield.

In all these coalfields coal is known to occur in the Damuda series. In the Hutar and Daltonganj coalfields the Barakar series is the coal-bearing formation and the Raniganj series is absent. In the Aurunga coalfield the deposits of the Raniganj series are also known to occur and these include a few minor coal seams. The workable coal seams in the Barakar series of the Aurunga field are known to be of a poor quality.

Fox (1934, p. 32) is of opinion that these coalfields are 'remnants of a much larger spread of Gondwana strata, and indeed were probably the westward extension of the Gondwanas of the Damodar Valley'.

A detailed account of the geology of this area is given by Ball (1878), who mapped the coalfield in the season 1877-1878. The coalfield was named as it is by him (*loc. cit.*, p. 4) after the village of Hutar. According to this author (*loc. cit.*, p. 91), the area of this coalfield is 78.6 square miles and the extent of different formations is as follows:—

Mahadeva series	..	14.1 sq. miles
Barakar group	..	57.0 "
Talchir	..	7.5 "

The Barakars overlap the Talchirs. The Mahadeva series is largely developed west of the Koel river and is not known to occur in the areas east of that river. Ball (1878, 1881) gives the thickness of Gondwana formations as follows:—

Mahadeva series	..	1,000 feet
Barakar	..	2,750 "
Talchir	..	300 "

Dunn (see Fox, 1934, p. 154) has estimated that the Mahadevas and Barakars are much smaller in thicknesses than given by Ball.

The Raniganj and Panchet series are known to occur in the eastern parts of the Aurunga field. The Raniganj series is underlain by the Barakars and the Panchet series is overlain by the Mahadevas. The Raniganj and Panchet series are absent in the Hutar field and the Mahadevas which are recognized in the western parts of this field rest directly and perhaps unconformably on the Barakars. In this connection Ball (1878, p. 48) observes: 'It may be that the absence of the normal sequence of beds between the Barakars and Mahadevas in the west may really be due to denudation'.

Ball (*loc. cit.*, p. 45) is of the opinion that the Mahadeva series in the coalfields of Karanpura to Tatapani which lie on the same line of strike have identical lithological characters and 'that these now detached areas are the remnant of a once continuous deposit'. But these deposits differ in their lithological characters from those in the more eastern fields of Bokaro and Raniganj. This author goes on to say: 'On the other hand, there is a very considerable resemblance between these rocks both structurally and lithologically, and those of the Hingir field, whose fossil contents have determined their age as belonging to the Kamthi-Raniganj groups'.

Faulting is not very extensive in this area and the rock formations have low angles of dip. There is a main fault running in an east to west direction and there are also smaller faults along the north-western and western ends of the field. Ball (*loc. cit.*, p. 52) has classified the faults in the Aurunga and Hutar fields into three groups of (1) east to west faults, (2) north of east to south of west faults, and (3) north of west to south of east faults. Two east and west faults of the first group, one fault of the second group forming the north-west boundary and a fault of the

third group forming the 'terminal western boundary' are recognized in the Hutar coalfield by this author.

Intrusion of dolerite has taken place in the Talchir formations.

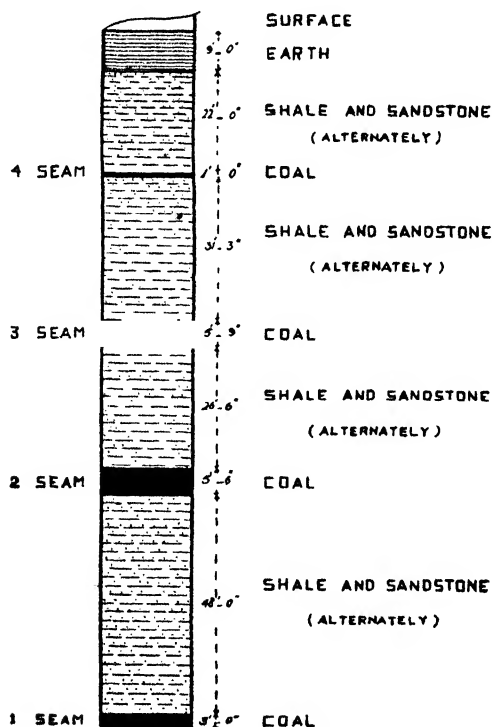
Coal seams

Dunn (see Fox, 1934, p. 155) has recognized five seams of coal and shaly coal along the Deori nala. Two seams, No. 2 and No. 3 from bottom, are being worked in the Hutar colliery. Messrs. Martin & Burn, Ltd., own this colliery and the note which follows is kindly supplied by the Manager of the colliery:—

'Two seams Nos. 2 and 3 are being worked in this colliery. Both the seams are outcropping on the south of the Deori River at the middle of the colliery lease hold. On the west the seams are traced up to the Koel River about three miles away from the colliery. On the east and south the exact extent is difficult to describe, because no exploration has been made to our knowledge. The mine is worked by means of inclines. Full dip $S10^{\circ}E$ at the 10 approximate. Output in 1953: 70,000 tons approximate.'

A section of the strata at the 23rd level of No. 3 seam as kindly supplied by the Manager of the colliery is shown in the figure below.

SECTION OF STRATA



The samples of coal described in this paper were collected from seams Nos. 2 and 3.

The results of the proximate analyses of a few typical samples of the two seams, kindly determined by Mr. H. S. Pareek, are given in the table on next page

Results of the proximate analyses of Hutar coals

S. No.	Seam	Proximate Analysis (per cent)				Calorific Value B.Th.U.		Volatile Coke Button	Colour of Ash	Colliery
		Moisture	Volatile Matter (less Moisture)	Ash	Fixed Carbon (by difference)	Volatile Matter	Fixed Carbon			
1	No. 2 (Sample 1)	6.88	38.44	4.81	49.87	43.53	56.47		Light buff	Sone Valley Hutar
2	No. 2 (Sample 2)	7.94	38.78	4.55	48.73	44.31	55.69	13,411	"	"
3	No. 3 (Sample 1)	6.37	50.26	8.11	35.26	58.77	41.23	"	"	"
4	No. 3 (Sample 2)	7.64	42.12	4.41	45.83	47.89	52.11	"	"	"

In hand specimen the Hutar coals are dull in appearance. The coal is composed largely of dull bands of durain including numerous narrow strips of vitrain which are up to 2 or 3 mm. in thickness; many of these, however, are less than 2 mm. thick. These vitrain strips do not follow a persistent course but are present only in short narrow bands thinning out at the ends and merging in the general mass of durain. Their presence in this manner gives a streaky appearance to durain. The occurrence of vitrain in thick persistent bands is a very uncommon feature in these coals. Fusain is found in great abundance and a block of coal may easily split along fusain layers and the surfaces thus exposed are seen to consist almost wholly of charred strips of fusinized wood which can easily soil the hand. On account of these facts the Hutar coals are dull in appearance and lack the characteristic banding into bright and dull layers so conspicuously exhibited by the coals of the nearby Damodar Valley coalfields. In this respect as also in the nature of their microscopic constituents described below, the Hutar coals seem to resemble more closely the coals of the far away Talcher and Pench Valley fields.

MICROSCOPICAL EXAMINATION

Microscopic study was made by an examination of thin sections in transmitted light and polished blocks in reflected light under oil immersion. Owing to the presence of a large proportion of high rank tissue like fusain and dark fungal bodies in these coals, their thin sections cannot be obtained easily and in the last stages of grinding most of this material is removed from the section. These constituents, however, reveal their structures in a clear manner on polished surfaces of coal. Low rank tissues like vitrain and many components of durain including spore material, resins and mineral matter can be examined in detail in thin sections.

A detailed examination of these coals has revealed the presence of well preserved and interesting structures in the different coal components. A major part of these coals is formed of micro-fragmental materials. Among the macro-fragmental components fusain and vitro-fusain are present in greater preponderance as compared to vitrain.

(a) Macro-fragmental coal

(1) *Fusain and Vitro-fusain*

Fusain is widely distributed in these coals and is easily distinguished in polished surfaces by its high reflectance showing it as the brightest component of coal. No attempt has been made to distinguish the various metamorphic stages of transformation from vitrain to fusain recognized by Seyler (1941, 1948), by measuring the reflectance of each step of transformation. The term vitro-fusain is used here in a wider sense including all the intermediates which may be present in these coals.

The structures in fusain and vitro-fusain tissues are shown in Plate XI, Figs. 1-6 and Plate XII, Figs. 1, 3 and 5. Plate XI, Figs. 1 and 2 show a transverse section of a woody tissue as seen in a polished surface. The pattern of cell walls appears white; the cell cavities are empty and appear dark. In Fig. 1, the cell walls are thin and show pits preserved in a clear manner. This fact leaves no doubt that we are dealing here with a woody tissue. The effect of pressure is apparent where the cell walls have undergone a slight folding. Fig. 2 shows more or less rectangular cells of a secondary woody tissue arranged serially and having comparatively thicker walls. Towards the left-hand side this structure passes into a band of thin-walled tissue similar to that in Fig. 1. This band, however, shows some broken cells as a result of pressure during coal formation; the band of secondary woody tissue having thicker walls has resisted this pressure. The cell cavities in both the bands are empty.

Plate XI, Figs. 3 and 5 show thick-walled woody tissues in fusain with empty cell cavities. On account of the fact that the tissues have yielded to pressure in different ways, the structures seen in the two figures appear somewhat different from each other.

In Fig. 5 there are blocks of thick-walled rectangular or rounded cells surrounded by broken and crushed mass of a similar kind of tissue. Fig. 3 shows a similar kind of picture with the difference that the cell walls in this tissue are comparatively thinner. In Fig. 3, the crushing of the cell walls in the central part has proceeded a step further and the broken and crushed mass is arranged in more or less a regular fashion. In this part the bogen structure which is characteristic in vitro-fusain is clearly seen. A similar structure is seen in Fig. 5 near the lower edge at the right-hand side.

Plate XI, Fig. 4 shows longitudinal section of a woody tissue revealing very well preserved bordered pits. The thick-walled wood fibres are folded and crushed at places and their cavities are empty. The walls of the fibres are seen to exhibit rows of bordered pits which have produced a chain-like structure. Plate XI, Fig. 6 shows the same kind of structure preserved in a clearer manner. This tissue, however, does not show any visible signs of crushing and the pits have produced a X-shaped chain in some fibres seen clearly in the central part.

A contact zone between two bands of a woody tissue in vitro-fusain appears in Plate XII, Fig. 1. On the left-hand side the section has cut the tissue longitudinally and the long thick-walled fibres of wood show bordered pits very clearly preserved; on the right-hand side the tissue is cut transversely and shows a mass of broken and crushed cells which have produced the characteristic bogen structure. The line of contact appears a rather sharp one.

A cellular tissue exhibiting some interesting features is shown in Plate XII, Fig. 5. There is a band of thin-walled tissue which is slightly compressed and as a result some cells are broken. A few cells have empty cavities. The cells generally show some opaque or semi-opaque material in their cavities. The cell contents have lower reflectivity as compared to the cell walls with the result that they do not appear equally bright. It is clear that these contents do not fill the cavities completely, but leave a narrow empty space which appears dark along their margins near the cell walls. In their outline they roughly resemble the general shape of the cell walls. The presence of these features makes it rather difficult to explain their origin. In all probability they represent some kind of a coal substance which was filling the lumen of the cells and has partly dried up in the process of coalification. These contents probably do not represent remains of plant fragments in the cell cavities. The presence of fragments of broken cell walls and of fine opaque and translucent granular matter in the lumen of cells was noticed in some Raniganj coals (Ganju, 1954). A characteristic feature of the fragmentary material is angular shape of its constituents, in contrast to rounded outline shown by the contents of the cells under discussion here. It is probable that the material was filling the cell cavity to its capacity in the initial stage and that it has gradually shrunk in the process of coal formation.

If the process of disintegration of the cell walls was complete in this tissue, the material forming the cell contents would be jumbled together and its identification would be a matter of doubt. It is possible, therefore, that the massive type of micrinite which forms an important constituent of durain in these coals has in part at least originated in this way from a similar kind of tissue.

Surrounding this tissue there are narrow bands of vitrain which appear less bright as compared to fusain and vitro-fusain materials and include round resin bodies. A well-preserved resin showing innumerable minute air bubbles appears near the edge in the upper left-hand side of this figure.

A thin section of fusain tissue in transmitted light is shown in Plate XII, Fig. 3. The cell cavities of a thick-walled woody tissue are seen here as rectangular white

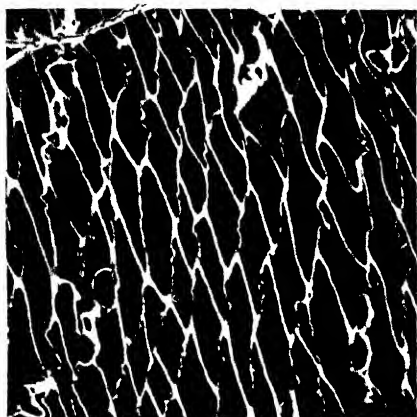


FIG 1 (X200)



FIG 2 (X200)

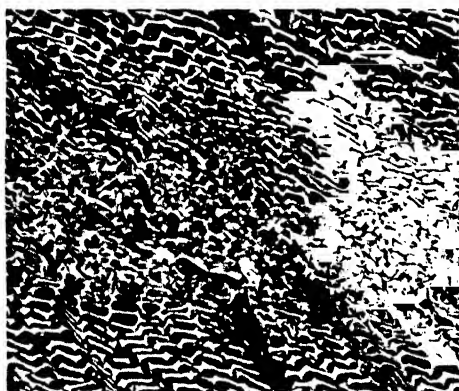


FIG 3 (X200)

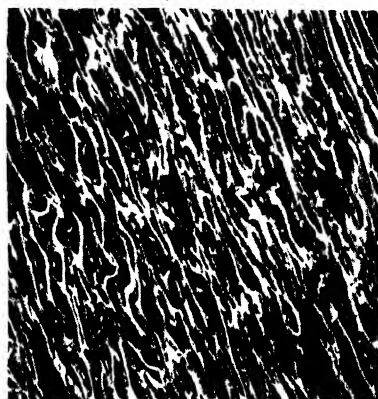


FIG 4 (X200)



FIG 5 (X200)



FIG. 6. (X200).



FIG. 1. (X200).



FIG. 2. (X200).



FIG. 3. (X400).

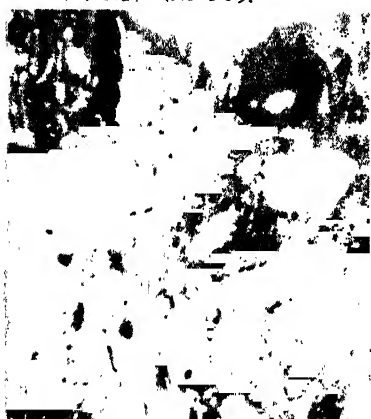


FIG. 4. (X100).



FIG. 5. (X200).

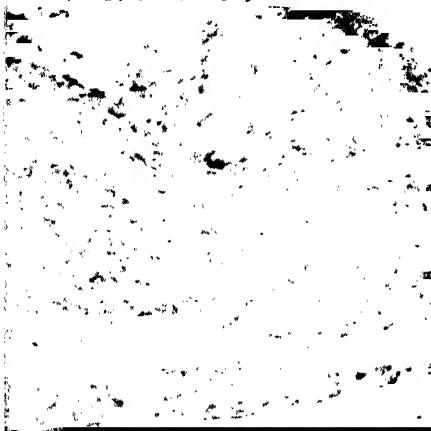


FIG. 6. (X100).



FIG. 7. (X400).

spaces. These are, however, not empty but filled with a mineral substance which is probably kaolinite. The cell walls do not transmit any light and are opaque. It is clear that this tissue does not show any visible signs of crushing on account of the fact that the cells are thick-walled and their cavities are filled with a mineral substance.

(2) *Vitrain*

It has been already indicated that the Hutar coals include only a few narrow irregular bands of vitrain which do not reveal any well-preserved structures, except some round or oval resin bodies. In reflected light vitrain tissue appears less bright as compared to fusain and vitro-fusain on account of its low reflectivity. This is clearly seen in Plate XII, Fig. 5 which shows a band of vitrain tissue in the upper left-hand side and a small portion of another band in the lower right-hand corner.

(b) Micro-fragmental coal

Durain

The durain in these coals shows a very variable composition. Its constituents observed in polished surfaces appear in Plate XII, Fig. 2 and Plate XIII, Figs. 2, 3 and 5-7, and those seen in thin sections are exhibited in Plate XII, Figs. 4, 6 and 7 and Plate XIII, Figs. 1 and 4.

A distinguishing feature of durain in these coals is the abundance of fungal bodies in different shape and forms and also of megaspores and microspores. The fungal bodies have caused a widespread decay of plant tissues. In addition to these dominant constituents, patches or rounded bodies of fusain, long narrow strips of vitrain which do not reveal any structure, fragments of wood fibres, micrinite, oval resins and mineral matter are abundant in durain. A detailed description of these constituents follows.

Spore exines

A detailed study of microspores in thin sections of coal is not possible on account of their very small size. They appear pale yellow or amber coloured, flatly compressed, short needle-like bodies scattered in the general mass of durain. Some durains may show microsporangia including closely packed microspores in large numbers. The microspores are probably studied in a better way by macerating the coal with Schultze solution. In this way the spores can be separated from the matrix and their surface features observed in minute detail.

Both seams No. 2 and 3 in the Hutar colliery show megaspore exines in large numbers. Plate XII, Fig. 7 and Plate XIII, Figs. 1 and 4 show some of these exines as seen in thin sections. Plate XII, Fig. 7 shows a peculiarly folded exine with its outer surface covered with a hair-like growth and having a small neck-like outgrowth at one end. Plate XIII, Fig. 4 shows two megaspore exines lying side by side embedded in an opaque material in a band of durain. Both these exines bear tuberculate outgrowths. The bigger spore on the lower left-hand side shows a wing-like appendage at one end. Plate XIII, Fig. 1 shows two megaspore exines, one in the centre near the upper edge and another near the lower edge in the right-hand side.

Plate XII, Fig. 2 and Plate XIII, Fig. 6 show two megaspore exines in durain as seen on polished blocks of coal. Both these exines bear tubercles on their outer surface and the exine in Plate XII, Fig. 2 shows also a neck-like attachment at the right-hand side. It is clear in Plate XIII, Fig. 6 that while nearly all the constituents of durain are prominently seen, the spore exine appears in the upper right-hand corner as a dark object not revealing any of its finer details.

The microspores in thin sections are seen in Plate XII, Figs. 4 and 6 and Plate XIII, Fig. 1 as short flatly compressed pale yellow bodies often with a crooked or an irregular crescent shape, scattered in the general mass of durain. In Plate XIII, Fig. 1 they appear as bright objects against the dark background of a highly carbonized ground mass of durain. A microsporangium-like body is seen in Plate XII, Fig. 6. Here a large number of small golden yellow bodies occur very closely packed together in durain. This object may be a torn portion of a compressed microsporangium. The sporangium was probably buried before it was ripe to burst and shed the spores. Similar objects are known to occur in the durains of Talcher coals.

Fungal bodies

A characteristic feature of these coals is presence of fungal bodies in large proportion. This fungal material usually appears dark in a thin section of coal, in the same way as do high rank tissues like fusain and vitro-fusain, and it is not easy to make the section any thinner for their examination in transmitted light. On the other hand, the fungal bodies show their features clearly in polished blocks. Their reflectivity approaches very near that of a high rank tissue in vitro-fusain.

Fungal remains and fungal spores have been described to occur in large numbers in the Tertiary coals of Assam and also in the Lower Gondwana coals of the Talcher coalfield (Ganju, 1954).

The main constituents of these fungal remains are sclerotia. They are hard and compact rounded bodies formed of a dense aggregate of interwoven hyphae and covered with a thick-walled outer coat of brown or blackish cells. They vary much in size and contain a good supply of reserve material. After remaining dormant for a considerable time they produce sporophores or conidiophores. The durain material round these hard ball shaped sclerotia is compressed very closely. It is generally noticed that these fungal bodies have destroyed the cellular structure of wood in coal in their vicinity.

Hacquebard (1952) has suggested that sclerotia which show no clear structure of the fungal hyphae may be termed 'sclerotoids' and when their structure is observed clearly the term sclerotium may be used.

The various types of fungal bodies in the Hutar coals are shown in Plate XII, Fig. 4 and Plate XIII, Figs. 2, 3, 5 and 6.

Plate XIII, Fig. 2 shows a fungal body resembling sclerotia. It is more or less oval in shape and has a mass of cellular structure surrounded by a hard solid ring. Another type of what is probably a fungal body showing a carved outline and some peculiar curved or straight markings on the surface is observed in Plate XIII, Fig. 5. These markings appear black and probably represent cracks caused by pressure on the outer hard shell of the body during the process of coal formation. In thin sections these cracks appear as white lines in the dark fungal body. This is observed in a fungal body in the upper right-hand side in Plate XII, Fig. 4. Similar objects have been described by Hacquebard (1950, 1952) under the term 'sclerotoids'. Plate XIII, Fig. 3 shows a rounded body probably of fungal origin. The material of durain surrounding these bodies has been closely compressed.

A small fungal body resembling sclerotia is seen embedded in the general mass of durain in Plate XIII, Fig. 6 at the upper left-hand side near the edge.

The fungal bodies in thin sections are shown in Plate XII, Fig. 4 which shows three dark bodies round or oval in shape, forming a major constituent of durain. On observing carefully it will be seen that the one near the upper margin shows a number of short straight or curved faint white lines which most probably represent empty cracks produced by pressure. These cracks have been referred to earlier in Plate XIII, Fig. 5. The fungal bodies are surrounded by thin and decomposed strips of woody vitrain. In all probability these strips are the decomposed products of a woody tissue which was destroyed by the agency of fungal organisms.

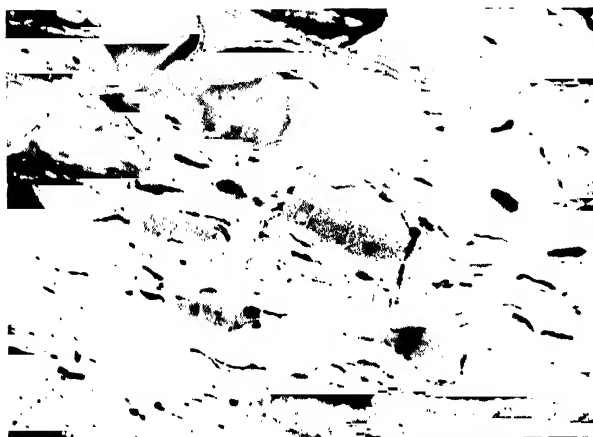


FIG. 1. (X100)



FIG. 2. (X200)



FIG. 3. (X200)



FIG. 4. (X90)



FIG. 5. (X200)

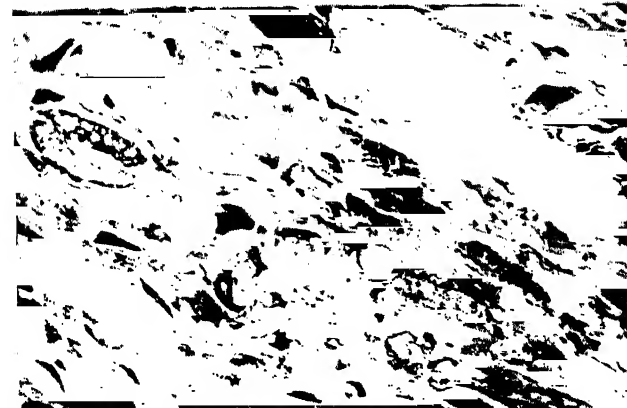


FIG. 6. (X200)



FIG. 7. (X200)

In addition to these fungal bodies and spore exines the durain contains a large quantity of vitrain material occurring in small bits or in thin elongated strips which do not reveal any structure. Fusain occurs in small rounded balls or may be dispersed in a broken state in the general mass of durain. Narrow bands of vitre-fusain tissues are also prominent in some durains. These features are clear in Plate XIII, Figs. 6 and 7. Small grains of quartz and kaolinite constitute mainly the mineral matter present in these durains. Resinous bodies are found to occur in an appreciable amount. In thin sections they appear rounded or oval and brownish red in colour resembling closely vitrain in its colour and transparency. Their form, however, readily distinguishes these bodies from vitrain components. The resinous material may be homogeneous or finely granular often showing air bubbles in large numbers. Plate XIII, Fig. 1 shows a number of oval resins filled with a more or less homogeneous material and showing some dark granular bodies in small numbers. These resins show a few cracks which are most probably formed during the process of section making. An oval resin showing innumerable minute air bubbles as seen on a polished surface appears in Plate XII, Fig. 5 near the edge; in the upper left-hand side.

Micrinite

The finely divided granular and coarse material which remains opaque in transmitted light and shows no traces of structure is included in micrinite. Finely granular micrinite has been described to occur in certain Indian coals (Ganju, 1954). While this variety is not observed in the Hutar coals, the massive type of micrinite, some of which is probably formed of bits of much decomposed and carbonized walls of wood fibres, is present in a fair amount in the durains of these coals.

SUMMARY AND CONCLUSIONS

A study of representative specimens of coal from seams No. 2 and No. 3 in the Sone Valley Hutar colliery was made with a view to ascertain the nature of microscopic constituents of these coals.

Examination in hand specimen reveals that these coals are composed largely of dull bands of durain including numerous narrow strips of vitrain which have produced a streaky appearance in the durain bands. Fusain is found in great abundance and a block of coal splits easily along the fusain layers revealing charred strips of fusinized wood on the surfaces thus exposed. Thick persistent bands of vitrain occur scarcely in the coals examined.

The results of proximate analyses of a few representative samples from the two coal seams show these coals to be generally lower in rank, as compared to coals of the Damodar Valley coalfields.

Microscopic study was made by an examination of thin sections in transmitted light and of polished surfaces in reflected light under oil immersion.

An examination of microscopic constituents has revealed that these coals are largely composed of woody tissues preserved as fusain and vitre-fusain which often show very well preserved cellular structures in reflected light. The cell walls are thick and the cell cavities usually empty. The effect of pressure is often evident where the cell walls are broken and crushed producing the bogen structure characteristic of vitre-fusain. Longitudinal sections of woody tissue have revealed bordered pits which have produced X-shaped chains in the walls of wood fibres. In certain woody tissues the cell cavities are partly filled with some opaque or semi-opaque material which has probably shrunk in the process of coal formation. In thin sections of some fusain bands the cell cavities appear filled with a mineral material resembling kaolinite.

Vitain tissues occur scarcely and when present do not reveal any structure.

The durain shows a variable composition and its two distinguishing features lie in the abundance of fungal bodies and megaspore exines. The fungal objects, some of which resemble sclerotia, have caused a widespread decay in the woody tissues. Fusain occurs in a finely dispersed state or in small rounded balls. Long narrow strips of vitrain are abundant. Microspores are very abundant. The durain also includes bits of broken cell walls of wood fibres, angular fragments of micrinite, oval resins, and grains of quartz and kaolinite.

In the nature and distribution of their microscopical constituents the Hutar coals resemble more closely the coals of Talcher coalfield than they do to those of the Damodar Valley coalfields. This fact may suggest that similar conditions of deposition were prevailing in the Hutar and Talchir basins.

ABSTRACT

The results of microscopical examination of coals from seams No. 2 and 3 in the Sone Valley Hutar coalfield are described in this paper.

In hand specimen these coals do not exhibit a well developed banding into bright and dull layers but are largely composed of dull bands of durain including numerous streaks of vitrain. Charred strips of fusain are exposed in abundance on surfaces parallel to bedding planes.

Examination of microscopic constituents in thin sections and on polished surfaces shows that these coals are largely composed of woody tissues preserved as fusain and vitro-fusain which reveal well preserved plant structures. Vitrain tissues occur scarcely and do not exhibit any cellular structure. The durain shows fungal bodies and megaspore exines in great abundance. Its fusain constituents occur in a finely dispersed state or in rounded balls. Fragments of cell walls of fusinized woody tissue, narrow strips of vitrain, angular pieces of micrinite, oval resins, flatly compressed microspores, and grains of quartz and kaolinite are other recognizable constituents in durain.

In their appearance in hand specimens and in the nature of their microscopic constituents these coals are more or less similar to the Talcher coals but are markedly different from coals of the Damodar Valley coalfields.

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DESCRIPTION OF PLATES

PLATE XI

(All figures are from polished surfaces in reflected light under oil immersion.)

- FIG. 1. Transverse section of a woody tissue preserved as fusain. The cell walls showing pits are thin and appear white in outline; the cell cavities are empty and appear dark. Some cell walls are slightly folded as a result of compression. No. 2 seam. ($\times 200$).

- Fig. 2. Rectangular cells of a secondary woody tissue in fusain arranged serially appear in the right-hand side. In the left-hand side this structure passes into a band of tissue similar to that in Fig. 1, but showing many broken cells. No. 2 seam. ($\times 200$).
- „ 3. Thick-walled woody tissue in fusain showing effects of compression. The cell walls are broken and in the central part they are crushed producing the characteristic bogen structure. No. 2 seam. ($\times 200$).
- „ 4. Longitudinal section of a woody tissue showing well preserved bordered pits, which have produced a chain-like structure in the walls of wood fibres. The fibres are broken at some places. No. 2 seam. ($\times 200$).
- „ 5. Transverse section of woody tissue in fusain showing blocks of thick-walled cells surrounded by a broken and crushed mass of cell walls which have produced bogen structure at some places. No. 3 seam. ($\times 200$).
- „ 6. Longitudinal section of a woody tissue similar to that in Fig. 4. The bordered pits have produced a X-shaped chain in some fibres. No. 2 seam. ($\times 200$).

PLATE XII

(Figs. 1, 2 and 5 are from polished surfaces in reflected light under oil immersion; Figs. 3, 4, 6 and 7 are from thin sections.)

- Fig. 1. A contact zone between two bands of a woody tissue in vitro-fusain. On the left-hand side thick-walled wood fibres showing bordered pits are shown in longitudinal section; on the right-hand side a mass of broken cells which have produced bogen structure at some places are seen in transverse section. No. 2 seam. ($\times 200$).
- „ 2. A megaspore exine showing a tuberculate outgrowth on the outer margin and a narrow neck-like attachment at the right-hand side. No. 3 seam. ($\times 200$).
- „ 3. Transverse section of woody tissue in fusain as seen in a thin section. The cell cavities which appear white are filled with a mineral substance probably kaolinite. No. 2 seam. ($\times 400$).
- „ 4. General view of durain showing three dark rounded or oval fungal bodies. The one near the upper margin shows a number of short straight or curved faint white lines which are probably cracks produced by pressure. The fungal bodies are surrounded by thin strips of vitrain. No. 3 seam. ($\times 100$).
- „ 5. Transverse section of a band of thin-walled cells which are slightly compressed. Generally the cells include some material filling partly their cavities. The cell contents are probably formed of an opaque or a semi-opaque coal substance which was initially filling the lumens completely but has partly dried in the process of coal formation. The cell contents have lower reflectivity as compared to the cell walls. An oval resin including minute air bubbles is seen near the edge in the upper left-hand side. No. 2 seam. ($\times 200$).
- „ 6. Part of a microsporangium-like body including a large clustre of microspores closely packed in durain. No. 2 seam. ($\times 100$).
- „ 7. A folded megaspore exine. The outer surface appears to be covered with a hair-like growth and at one end in the upper part there is a small neck-like outgrowth. No. 3 seam. ($\times 400$).

PLATE XIII

(Figs. 1 and 4 are from thin sections: Figs. 2, 3, 5, 6 and 7 are from polished surfaces in reflected light under oil immersion.)

- Fig. 1. Durain showing a number of oval resin bodies filled with a more or less homogeneous material and showing dark granular bodies in small numbers. Two megaspore exines stand prominently in durain which also includes microspores in large numbers. The microspores appear as pale yellow, flatly compressed, short needle-like objects often with a crooked shape. No. 3 seam. ($\times 100$).
- „ 2. An oval fungal body resembling sclerotia. It shows a mass of cellular tissue surrounded by a solid ring. No. 2 seam. ($\times 200$).
- „ 3. A rounded body of a probable fungal origin. The surrounding material of durain is closely compressed. No. 2 seam. ($\times 200$).
- „ 4. Two megaspore exines showing tuberculate outgrowth are lying side by side in fusinized material in durain. The bigger exine at the left-hand side shows a wing-like appendage at one end. No. 2 seam. ($\times 90$).
- „ 5. An oval body probably fungal in origin, showing a carved outline and peculiar curved or straight markings on the surface. These markings may be due to cracks caused by pressure during coal formation. In thin sections these cracks appear as faint white lines as shown in Plate II, Fig. 4. No. 2 seam. ($\times 200$).

- Fig. 6. Durain showing its constituents clearly preserved. A small oval fungal body resembling sclerotia is seen in the upper left-hand side. A megaspore exine is preserved in the upper right-hand corner. Short narrow bands of vitrain and vitro-fusain tissues, finely divided and rounded fusain material, micrinite and grains of quartz and kaolinite are other characteristic constituents of this durain. No. 2 seam. ($\times 200$).
- „ 7. General view of constituents of durain showing thin elongated strips of vitrain and vitro-fusain tissues, broken walls of wood fibres, some massive type of micrinite and spore exines. No. 3 seam. ($\times 200$).

Issued November 23, 1955.

CONTRIBUTIONS TO THE CYTOLOGY OF HYMENOMYCETES

I. CYTOLOGICAL STUDIES IN *MARASMIUS CAMPANELLA* HOLTERM.

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INTRODUCTION

Cytological investigations on Hymenomycetes in India has so far been a minor feature. It was Bose (1937) who first made an attempt in this direction only with eleven species of polypores and showed a great deal of consistency in karyogamy being subsequently followed by meiotic divisions in the basidial development. So far as the authors are aware, no further work has been done in India either on other members of Polyporaceae or those of allied families which will be evident from Olive's (1953) excellent review on the subject. In order to throw additional light on nuclear phenomena in other members of Hymenomycetes in India, investigations are now in progress of which the present paper forms the first contribution to the series.

While studying the biology of *Marasmius campanella* Holterm., it was found by the writers (1954a) that the fungus is homo-heterothallic. In order to determine what cytological basis underlies this phenomenon and what light it throws on the life-cycle of the fungus, the present investigation was carried out.

MATERIALS AND METHODS

For the study of the hymenium, pilei of fresh fruit-bodies of *M. campanella*, at all stages of development were collected from Calcutta, cut into small pieces and fixed in several killing and fixing fluids. Of these, Bouin-Allen, Flemming's fluid (weak) and acetic-formalin-alcohol were found to be satisfactory. The materials were fixed at different intervals of time, viz., at 12 noon, and at 3 and 6 p. m. After fixation, washing, dehydration and imbedding were done in the usual way. Sections were cut and those 8–10 μ in thickness were found to be most satisfactory. Several stains, such as Heidenhein's iron-haematoxylin, aqueous basic fuchsin, i.e., Feulgen's method (1924) and aqueous crystal violet were tried, of which Heidenhein's iron-haematoxylin yielded the most satisfactory result. It is to be noted that for proper staining with iron-haematoxylin both mordanting and staining for 25–30 minutes were found quite suitable. For studying the nuclear conditions in spores, spore-deposit from fresh fruit-bodies were taken on clean grease-free sterilized slides. These were then fixed directly for 24-hours in Flemming's fluid and finally stained with iron-haematoxylin. Knip's (1913) agar-film technique and that modified by Sass (1929) was tried for making total preparations in order to study nuclear phenomena of spore-germination and in the mycelium. For studying spore-germination, spores from fresh fruit-bodies were allowed to drop on the agar-film on the slide and extreme aseptic precautions were taken to avoid contamination. The cultures at the desired stage of development were fixed and stained by the

methods already mentioned. Prior to fixation the slides were, however, transferred to dry Petri-dishes and the thin films of the medium were allowed to dry down only at the margin in order to avoid washing out of the films from the slides during the long process of washing and staining. In the agar-film technique, the film of the medium although stained deeply could be destained readily. The degree of destaining of the agar-film afforded a convenient guide to progress of differentiation.

OBSERVATION

The nuclear condition in this species may well begin with the study of the hymenium. The basidia are more or less clavate, disterigmatic, bisporous and each bears a clamp-connection at its narrowed base (Fig. 1, *r-s*). When young they are distinctly binucleate (Fig. 1, *a-c*). This is best demonstrated in certain cases when the two nuclei have been found about half-way up the basidium lying side by side in the general mass of cytoplasm (Fig. 1, *c*). Karyogamy occurs after this stage (Fig. 1, *d-e*) and the fusion nucleus moves towards the upper part of the basidium. The fusion nucleus is conspicuously large, homogeneous and well defined. Its nucleolus is brightly stained and the chromatin-reticulum is not in evidence. The chromosomes have, however, been demonstrated in the meiotic stages (Fig. 1, *f-h*) along with other stained granules in the cytoplasm and the number is $10 (= 2n)$. The presence of synkaryons following karyogamy clearly indicates that the basidial nuclei are in the diploid condition. Two successive nuclear divisions of the fusion nucleus take place in the basidium and four daughter nuclei have been found which seem to be alike in size, structure and staining properties (Fig. 1, *f-s*).

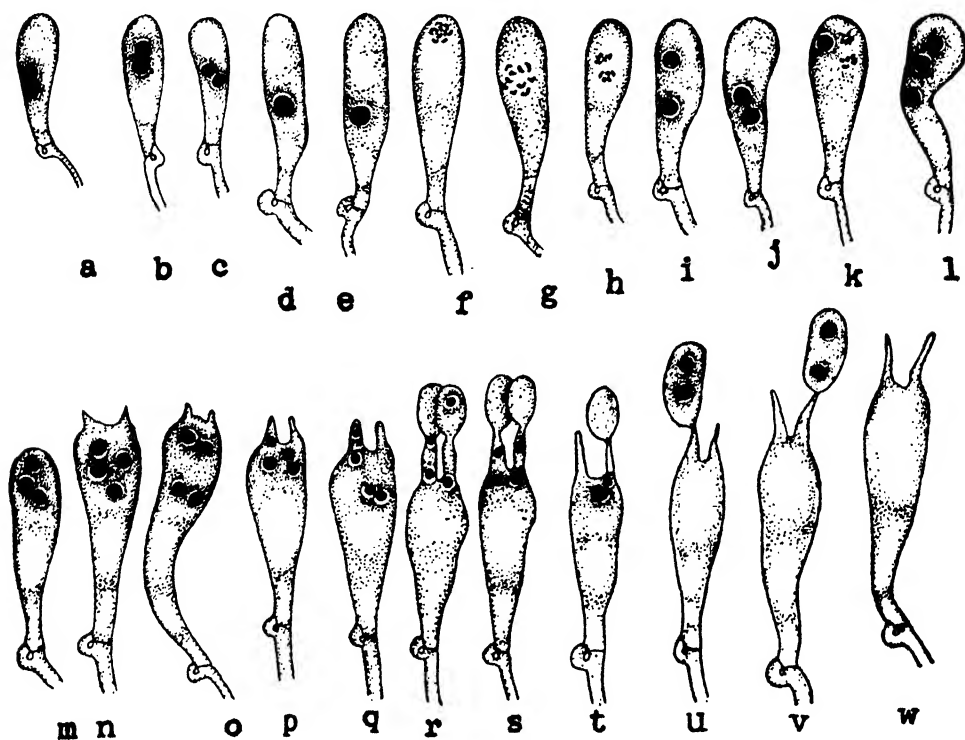


FIG. 1. Successive stages in the development of basidia and the associated nuclear phenomena. ($\times 2240$).

Migration of the daughter nuclei into the spores has been clearly demonstrated but the details of the procedure could not be observed (Fig. 1, *r-v*). Prior to the formation of sterigmata, the four nuclei within the basidium do not show any definite orientation. As the sterigmata and the spores develop, the nuclei, no doubt, move upward within the basidium, but at no time do they become attached to its upper wall (Fig. 1, *n-p*). Nuclei in pairs then prepare to undergo migration, one nucleus of each pair moving into the broad base of each sterigma (Fig. 1, *p*). In some cases, a pair of nuclei, one following the other, have been observed at the base of a sterigma and the other two nuclei are to be found at some distance away from it (Fig. 1, *q*). Subsequently, one nucleus has been found to complete its migration into a spore, while a second nucleus is found at the base of the same sterigma (Fig. 1, *r*). Usually, when all the daughter nuclei migrate into the spores, the basidium becomes empty

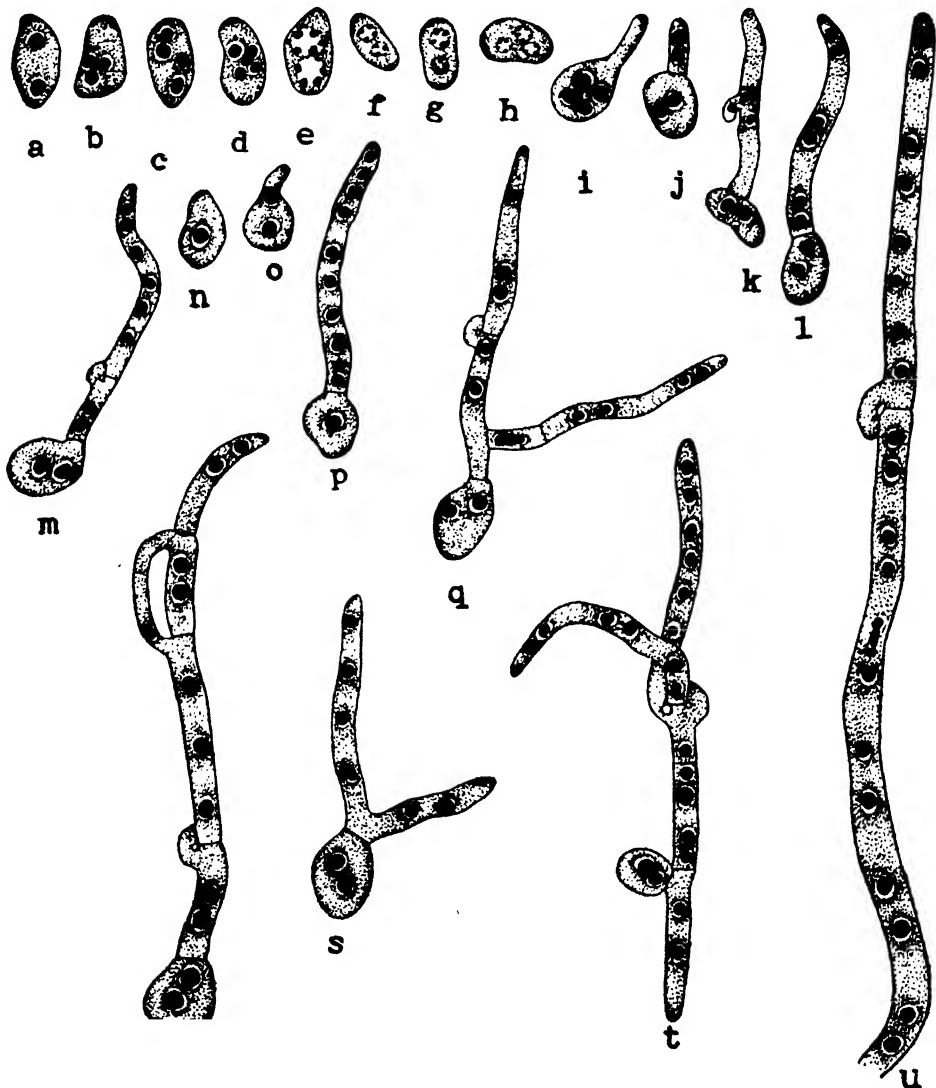


FIG. 2. Nuclear phenomena of spore-germination and in the mycelia. ($\times 2240$).

(Fig. 1, *u-w*). Each spore, when young, contains two nuclei in great majority of cases (Figs. 1 and 2, *u-v*; *a*), although a few uninucleate spores have been found also in a total preparation after the spores are discharged from the sterigmata (Fig. 2, *n*). In a properly fixed and stained preparation it has been found that the mature spores occasionally contain three or four nuclei (Fig. 2, *b-d*). Following Pinto-Lopes (1949) the nuclei of the spores can be grouped into two main types, viz., (i) homogeneous (Fig. 2, *a-d*) and (ii) disperse-expanded (Fig. 2, *e-h*). In most cases mycelia developing from single spore show clamp-connection, which appears early, usually, at the very first septum of the germ-tube (Fig. 2, *k, m, q-r, t-u*). Frequently, two germ-tubes may develop from any point of the spore-wall, but only one of them soon takes the lead (Fig. 2, *s-t*). In early stages of germination, when the germ-tube is first formed, the two pre-existing nuclei of the spore divide once forming four daughter nuclei (Fig. 2, *i*). Of these, two nuclei migrate into the germ-tube, while other two remain within the original spore-case (Fig. 2, *j*). The two nuclei of the germ-tube are eventually separated from the spore by the formation of a transverse septum at the junction of the germ-tube and the spore-wall. The formation of the first septum may be delayed for some time till the germ-tube attains a considerable length. As the germ-tube elongates, its two nuclei either divide by conjugate division forming clamp-connections (Fig. 2, *k*) and this process is repeated to form regular binucleate cells or the nuclei simply increase in number by irregular divisions and without the formation of any clamp-connection (Fig. 2, *l, m, q, u*). In the latter case, the largest number of nuclei in the cell of the germ-tube may be up to ten. Each nucleus occupies almost the entire width of the hypha, and its nucleolus stains intensely with iron-haematoxylin. Each uninucleate spore, on the other hand, at the time of germination produces the germ-tube in the form of a protrusion and the pre-existing nucleus divides once (Fig. 2, *o*). One of the nuclei then migrates into the germ-tube and the other remains within the spore-case. The germ-tube with one nucleus now elongates and soon a transverse septum is formed at its base near the junction of the spore-wall. Eventually, a primary mycelium is formed. Sometimes, wall-formation following nuclear divisions appears to be somewhat delayed (Fig. 2, *p*).

DISCUSSION

Since *Marasmius campanella* is homo-heterothallic, it will be of interest to discuss salient cytological features of this agaric. The spores are usually in the binucleate condition, but occasionally they may be uninucleate or contain more than two nuclei. The uninucleate condition may be interpreted as due to migration of a single nucleus from the basidium to the spore while trinucleate or quadrinucleate conditions appear to have originated due to subsequent divisions of the pre-existing nuclei within the spore prior to germination. In case of the spores that receive one nucleus only, the fate of the remaining nuclei in the basidium could not be followed clearly. The clamp-bearing mycelium is undoubtedly in the secondary condition and as the mycelium is of monosporous origin, the species is considered to be homothallic. However, the cells of the secondary mycelium are typically dicaryotic and with clamp-connections although three to seven nucleate hyphal cells with plain septa are not uncommon. This shows that the members of the conjugate nuclei can divide independently even when there is no question of clamp-connection. In the monocaryotic mycelium arising from uninucleate spores, the hyphal cells are not always associated with the formation of septa.

The basidia have always been found to be disterigmatic and bisporous. As usual, they are binucleate when young but the two nuclei soon fuse to form a synkaryon in preparation for the inception of meiosis. After meiotic divisions four daughter nuclei are formed of which usually two nuclei enter into each spore. In some cases, each of the two spores of a basidium receives a single nucleus. The

distribution of sex-factors and the consequent sex-reactions in this agaric can be explained on the basis of nuclear phenomena. It has been observed that a binucleate spore when germinates always gives rise to a typical dikaryotic mycelium with clamp-connections and from this mycelium normal fructifications are formed as reported by the authors (1954b). Occasionally, when a single nucleus enters into a basidiospore, it or its descendants possibly carry one sex-factor and this type of spore gives rise to exceptional primary mycelium. In such cases, secondary condition arises by hyphal fusions among primary mycelia of opposite-sex, and once the secondary condition is established, the nuclear cycle appears to be the same in all cases. It may be assumed that each binucleate spore, on the other hand, receives a compatible pair of nuclei from the basidium and is evidently in a dikaryophasic condition and must carry a complete set of opposite sex-factors. Increase in the number of nuclei in such a spore is purely vegetative.

SUMMARY

1. *Marasmius campanella* is an agaric with disterigmatic two-spored basidia and there is an alternation of diplophasic and haplophasic nuclei, the latter condition being represented by the haploid nuclei of the basidia following meiosis and those in the cells of dikaryotic mycelia. Karyogamy occurs in the basidium to form a fusion nucleus and the diploid condition is restored. This nucleus divides soon meiotically to form four haploid nuclei.

2. Each basidiospore normally receives two nuclei which may or may not divide again. Occasional spores are found to receive one nucleus. The uninucleate spores give rise to haploid mycelia with uninucleate or multinucleate hyphal cells which in order to produce secondary mycelium, fuse with one another in a limited number of combinations.

3. Early stages in the development of secondary mycelium show the presence of clamp-connections even at the first septum of the germ-tube or they are formed very soon thereafter. The clamp-connections are, however, not present at every septum, the same hypha bears both plain septa and septa with clamp-connections. Further, the cells of the secondary mycelium are not strictly dikaryotic since cells containing more than two nuclei are not uncommon.

4. Chromosomes have been found in pre-meiotic, meiotic and post-meiotic stages, the diploid number being ten and this represents the largest number discovered so far in Hymenomycetes.

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A PRELIMINARY REVIEW OF THE GENERA OF THE FAMILY BAGRIDAE (PISCES: SILUROIDEA)

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INTRODUCTION

The family Bagridae (type-genus *Bagrus* Cuvier) comprises cat-fishes distributed in the Ethiopian, Oriental and Palaearctic regions. These are primarily freshwater forms, though some species of *Mystus* Scopoli live in brackish waters also. No comprehensive treatise exists on the family as a whole, but certain genera have been revised piece-meal by various workers, such as Regan (1913, *Leiocassis* Bleeker), Rendahl (1928, south Chinese genera) and Worthington and Ricardo (1937, *Chrysichthys* Bleeker in part). An attempt is made here to review in a preliminary way the present systematic position of the various genera included under the family.

The work has been aided by the award of a research fellowship by the National Institute of Sciences of India, Delhi, to which the writer is much indebted. Besides, many foreign museums and ichthyologists have helped by lending material preserved in their museums for this study. Particularly, I wish to thank in this connection Drs. E. Trewavas, L. P. Schultz, S. L. Hora, G. S. Myers, T. Mori and Mr. J. T. Nichols.

NOMENCLATURE AND CLASSIFICATION

Fishes of this family have in common with other Siluroids barbels and a scaleless body which led earlier workers like Belon (1553), Artedi (1758), Linnaeus (1758) to name them as *Mystus*. The last author in his subsequent edition of the *Systema Naturae* kept them under the genus *Silure*. Bloch and Schneider (1801) considered them under the genus *Silurus*. Lacépède (1803) divided *Silure* into two groups, *Pimelodus* and *Doras*, the former for fishes with a nude body and the latter for those with a row of shields along its lateral line. Cuvier (1817) was the first to use the name *Bagres* as a separate taxon and he assigned *Silurus bayad* Forskål as the first species under *Bagres*. Hamilton (1822) adopted Lacépède's classification and described a number of these fishes under *Pimelodus*. Valenciennes (1849) adopted Cuvier's nomenclature but named the genus as *Bagrus* instead of the polynomial phrase *Les Bagres*. Bleeker (1862) gave a separate rank of 'Strips Bagrini' for these fishes and described a number of new genera under it. Günther (1864) included these fishes under his fourth subfamily Siluridae Proteropterae and gave a separate rank of 'group Bagrina'. Day (1878) did not recognize Günther's grouping but described all Siluroid fishes under the family Siluridae. He adopted almost the same classification in his *Fauna* volumes on fishes (1889, p. 101).

In 1911, Regan gave a separate family rank Bagridae to these fishes and divided them on the basis of osteological features into two subfamilies Chrysichthinae and Bagrinae. Jordan (1923), however, did not recognize these subdivisions but only listed a number of homonyms and synonyms under Bagridae. Fowler (1936, 1949) used the names Porcidae and Mystidae (1935) for Bagridae without assigning any

reasons whatsoever. Berg (1940) also followed Jordan, but did not list the genera. He merged Fowler's homonyms under Bagridae.

STATUS OF GENERA

A. FOSSIL.—Jordan (1923) listed under Bagridae 44 living and 4 fossil genera. Of the four fossil genera, *Glyptocephalus* Agassiz is stated to be a synonym of *Bucklandium* König (as per Jordan, 1919) which itself is said to belong to Tachysuridae in view of the 'inferior process at the junction of the basioccipital with the vertebral column which is diagnostic of the Aridae' (Regan, 1922). The remaining two fossil genera are *Fajumia* Stromer and *Socnopaea* Stromer. Peyer (1928) discussed the validity of these two genera and placed them under Tachysuridae.

After Jordan, White (1934) described *Eomacrones*, *Eaglesomia* and *Nigerium* from Nigeria which are valid.

Amongst the living genera, *Chrysichthys* Bleeker, *Mystus* Scopoli and *Rita* Bleeker are known to occur in the fossiliferous Siwalik beds of the Panjab in India. Hora and Menon (1953), on the basis of Woodward (1901) and Lydekker (1886), gave the range of time distributions of *Mystus* to Eocene Africa and of *Chrysichthys* to fossiliferous Pliocene Siwalik beds. Romer (1947), however, did not include *Chrysichthys* but listed *Macronoichthys*, *Heterobagrus* and *Bagrus* besides the above-named genera. The record of *Chrysichthys* from India, is based on Lydekker (1886), who himself was in doubt regarding the generic status of the specimen. The enlarged supraoccipital and post-temporal bones which are rugose appear to be those of *Rita* rather than of *Chrysichthys*.

Thus at present, the following genera can be stated to be known from the fossiliferous beds:—

- | | | |
|--------------------------------|----|-------------------------------|
| 1. <i>Eaglesomia</i> White | .. | Eocene, Africa. |
| 2. <i>Eomacrones</i> White | .. | Eocene, Africa; Recent, Asia. |
| 3. <i>Nigerium</i> White | .. | Eocene, Africa. |
| 4. <i>Bagrus</i> Cuvier | .. | Pleistocene—Recent, Africa. |
| 5. <i>Rita</i> Bleeker | .. | Pliocene—Recent, Asia. |
| 6. <i>Mystus</i> Scopoli | .. | Tertiary—Recent, East Indies. |
| 7. <i>Heterobagrus</i> Bleeker | .. | Pliocene—Recent, Asia. |

B. LIVING.—As stated earlier, Jordan (1923) listed 44 genera under Bagridae. They can be analysed as below.

I. The following genera do not now belong to Bagridae:—

<i>Olyra</i> McClelland	at present belongs to Family	Olyridae.
<i>Macronoides</i> Hora	Sisoridae.
<i>Laguvia</i> Hora	Sisoridae.
<i>Liobagrus</i> Hilgendorf	}	Amblycipitidae.
<i>Branchiosteus</i> Gill		
<i>Neobagrus</i> Bellotti		
<i>Cranoglanis</i> Peters		Cranoglanidae.

II. The following are synonymous with genera cited against each:—

Synonyms	Valid genera
<i>Mystus</i> Gronow, 1763	} <i>Mystus</i> Gronow (emend) Scopoli.
<i>Macrones</i> Dumèril	
<i>Aspidobagrus</i> Bleeker	
<i>Hypselobagrus</i> Bleeker	
<i>Aoria</i> Jordan	
<i>Hemibagrus</i> Bleeker	

Synonyms	Valid genera
<i>Auchenaspis</i> Bleeker	<i>Auchenoglanis</i> Günther
<i>Pseudobagrichthys</i> Bleeker	<i>Bagroides</i> Bleeker
<i>Rhinobagrus</i> Bleeker	<i>Leiocassis</i> Bleeker
<i>Porcus</i> Geoffroy	<i>Bagrus</i> Cuvier
<i>Melanodactylus</i> Bleeker	<i>Chrysichthys</i> Bleeker
<i>Chrysobagrus</i> Boulenger	
<i>Octonematichthys</i> Bleeker	<i>Clarotes</i> Kner
<i>Gogrius</i> Day	<i>Rita</i> Bleeker
<i>Pseudobagrus</i> Bleeker, 1860	<i>Pseudobagrus</i> Bleeker, 1858
<i>Fluvidraco</i> Jordan and Fowler	<i>Pelteobagrus</i> Bleeker

III. Besides the above cited 10 genera which are valid, the following 11 listed by Jordan are at present referable to Bagridae:—

<i>Rama</i> Bleeker	<i>Phyllonemus</i> Boulenger
<i>Bagrichthys</i> Bleeker	<i>Parauchenoglanis</i> Boulenger
<i>Heterobagrus</i> Bleeker	<i>Liauchenoglanis</i> Boulenger
<i>Gephyroglanis</i> Boulenger	<i>Amarginops</i> Nichols and Griscom
<i>Notoglanidium</i> Günther	<i>Gnathobagrus</i> Nichols and Griscom
<i>Leptoglanis</i> Boulenger.	

Since Jordan's time, Mori (1936) described *Coreobagrus* which is probably synonymous with *Leiocassis*; Poll (1942) described *Lophiobagrus* and the writer (1955a) *Horabagrus* which are valid. Thus at present, there are 23 genera under Bagridae which are reviewed in the following pages.

AFRICAN GENERA

1. **Bagrus** Cuvier—*Regne Animal.*, II, 1817, 204 (generic type by original designation *Bagrus bayad* Forskål).

This is the type genus of the family Bagridae. Fishes of this genus are very generalized and resemble to a great extent the species of the Indian genus *Mystus* Scopoli. There appears to be enough justification for using the name *Porcus* Geoffroy, an earlier name, though the familiarity of *Bagrus* is greater and appealing for its retention. Fowler (1936, p. 309) used the name Porcidae apparently on this basis, although he gave no reasons. The problem is being investigated. So far 10 species are known of this genus.

2. **Chrysichthys** Bleeker—*Acta. Soc. Sc. Indo-Neerl.*, IV, 1858, 60 (generic type by subsequent designation *Pimelodus auratus* Geoffroy).

This genus is in need of a complete revision. So far 40 species are known. Worthington and Ricardo (1937, p. 1089) found the continuous or discontinuous nature of the vomeropterygoid dentition of diagnostic value. Along with this, the occipital region of the head being covered or uncovered with skin, and the length-width ratio of the premaxillary band of teeth appear to be of taxonomic value. Many species seem to have been wrongly identified. This genus is also one of the generalized Bagrids, known even from fossiliferous beds. The Indian genus *Rita* Bleeker is allied to *Chrysichthys* in having small, slender, valve-like nasal barbels and in other general features. However, both are different in the character of palatine dentition and fin structure. An anatomical comparison is being undertaken.

3. **Clarotes** Kner—*Sitzb. Akad. Wiss. Wien.*, XVII, 1855, 313 (generic type by original designation and monotypy *C. heuglinii* = *Pimelodus laticeps* Rüppel).

This genus is closely allied to *Chrysichthys* excepting that besides the first dorsal fin the adipose fin is also modified into cartilaginous rays and ossified spines. This condition is met with only in adult specimens as young ones show absolutely no trace of any such modification (see Hyrtl, 1859 for detailed anatomical study). Whether on this basis, the fish should be given a generic distinction is questionable. However, for taxonomic purposes, external well-defined characters as these seem to be sufficient for a generic recognition. Two species are known at present.

4. **Gephyroglanis** Boulenger—*Ann. Mus. Congo belge.*, I, 1899, 42 (generic type by original designation *G. congicus*).

This genus is also allied to *Chrysichthys*, chiefly distinguished from it by the absence of teeth on the palate and rudimentary or sometimes absent nasal barbels. Ten species have so far been described of this genus.

5. **Phyllonemus** Boulenger—*Trans. Zool. Soc. Lond.*, XVII, 1906, 552 (generic type by original designation and monotypy *P. typus* Boulenger).

Worthington and Ricardo (*op. cit.*, p. 1096) emended the generic diagnosis by correcting Boulenger's statement that the prevomer alone is denticulous. They found after examining a large series of fresh specimens collected by Christy, that the pterygoids are also with teeth. The genus is allied to *Chrysichthys* from which it differs in having no nasal barbels, and the maxillary barbels fringed on both sides by a leaf-like membrane in its distal part. Whether this latter character is taxonomically significant is doubtful. Worthington and Ricardo (*loc. cit.*) examined specimens in which the distal part of the maxillary barbels is not leaf-like but flattened. Examination of a large series of specimens seems desirable, particularly of different age groups to find out any possible variations in respect of these two features.

6. **Auchenoglanis** (Günther)—*Cat. Fish. Brit. Mus.*, V, 1864, 137 (substitute name for *Auchenaspis* Bleeker preoccupied; generic type by original designation *Pimelodus biscutatus* Geoffroy).

7. **Parauchenoglanis** Boulenger—*Cat. African Fish. Brit. Mus.*, II, 1911, 364 (generic type by original designation *Pimelodus guttatus* Lonnberg).

Fourteen species are known of the first genus and three of the second. The generic limits of *Auchenoglanis* have been confused because of referring species such as *A. iturii* Steindachner, *A. büttikoferi* Popta, etc., which are referable to *Parauchenoglanis* in view of their having the teeth on jaws arranged in bands instead of in patches and having simple, non-fleshy lips. Similarly certain species such as *A. balayi* (Sauvage), etc., are different from *Auchenoglanis sensu stricto*. The latter category appears to be intermediate between *Auchenoglanis* and *Parauchenoglanis*.

8. **Leptoglanis** Boulenger—*Ann. Mus. Congo belge.*, II, 1902, 42 (generic type by original designation *L. xenognathus* Boulenger).

The affinities of this genus have so far been considered with Amphilidae by David and Poll (1937) and others. Examination of a specimen of *L. rotundiceps* (Hilgendorf) shows this contention to be an error. Harry (1953) in his revision of Amphilidae stated that *Leptoglanis* is a Bagrid by virtue of its possessing a normal,

large, free air-bladder. It seems likely that *Leptoglanis* gave rise to Amphiliidae in Africa and Amblycipitidae in Asia. Four species are so far known of this genus.

9. **Lophiobagrus** Poll—*Rev. Zool. Bot. afr.*, XXXV, 1942, 318 (generic type by original designation and monotypy *L. lestradei* Poll).
10. **Amarginops** Nichols and Griscom—*Bull. Amer. Mus. nat. Hist.*, XXXVII, 1917, 713 (generic type by original designation and monotypy *A. platus* Nichols and Griscom).
11. **Gnathobagrus** Nichols and Griscom—*Ibid.*, XXXVII, 1917, 711 (generic type by original designation and monotypy *G. depressus* Nichols and Griscom).

Each of these monotypic genera is known by their respective type specimens and has not been subsequently recorded. The genera appear to be primitive and interrelated. *Lophiobagrus* appears to be intermediate between *Amarginops* and *Gnathobagrus* and is perhaps only subgenerically distinct from the former. These genera appear to have given rise to *Chaca*-like fishes of India, Burma, Siam and Malaya through *Lophiosilurus*-like genera.

12. **Notoglanidium** Günther—*Proc. Zool. Soc. Lond.*, II, 1902, 336 (generic type by original designation *N. walkeri* Günther).
13. **Liauchenoglanis** Boulenger—*Cat. African Fish. Brit. Mus.*, IV, 1916, 314 (generic type by original designation and monotypy *L. maculatus* Boulenger).

These two genera are peculiar. The first one is known by two species *N. walkeri* Günther and *N. thomassi* Boulenger, while the second is monotypic. Both are different from all the other Bagrids in having a long rayed dorsal fin of 14 to 20 rays, no occipital process and a very low, posteriorly adnate, small adipose dorsal fin extending to the caudal fin. On this combination of external characters alone, it seems justified to keep them under a separate subfamily Notoglanidini.

INDIAN GENERA

14. **Rita** Bleeker—*Ichth. Archipel. Ind. Prodr.*, I, *Siluri*, 1858, 60 (generic type by original designation *Pimelodus rita* Hamilton = *Rita buchmani* Bleeker).

This is the only Bagrid genus known so far with 7-8 rays in the pelvic fin (*versus* uniformly 6 in all others); three pairs of barbels, maxillary, nasal and only one pair of mandibulars (*versus* two pairs of mandibulars in genera with three pairs of barbels) and prevomer enlarged and disc-shaped (*versus* simple). In order to find whether these features are constant in all the species and in all the individuals of each species, a tour to the Mahanadi, Godavari and Krishna river systems was undertaken. About 400 specimens of *Rita chrysea* Day, previously known only from 13 specimens; about 275 of *R. hastata* (Valenciennes); about 40 of *R. pavimentata* (Sykes) were collected. About 60 specimens of *R. rita* (Hamilton), the generic type, were also examined. In none of the specimens of the four species examined the pelvic fin ray count is found to be six, but only either seven or eight. As such, *Rita* is kept under a separate subfamily Ritaini. Osteological comparison with other available genera is in progress.

15. **Rama** Bleeker—*Atlas Ichth. Ind. Orient.*, II, 1862, 8 (provisionally proposed; generic type by original designation and tautonymy *Pimelodus rama* Hamilton = *Rama rama* Bleeker).

No material of *P. rama* Hamilton and *P. chandramara* Hamilton, the two species of this genus so far known, is stated to exist in any museum. Bleeker provisionally erected this genus and included it under the Phalanx 'Ritae' along with *Rita*. The original drawings and illustrations of the two species show clearly that the former is sufficiently distinct to warrant a new generic status and that the latter is only questionably separable from the known species of *Rita*. There are discrepancies in Hamilton's description of these species between his original MS. and his published account in the *Gangetic Fishes*. Day's specimen from Assam, *Leiocassis rama* is distinctly referable to *P. rama* as shown by an examination of the much damaged specimen of the former species, present in the ZSI. Day and Regan (1913) were wrong in considering this species as belonging to *Leiocassis* which does not occur in India.

16. **Mystus** Scopoli—*Introductio ad historiam*, 1777, 451 (generic type by subsequent selection *Silurus pelusius* Solander).

The nomenclatural status of this genus has been elucidated in another article (Jayaram, 1955b). This genus is very generalized, with 39 species, from which many forms seem to have arisen. It seems likely that several subgeneric complexes are present in this widely distributed genus. *Heterobagrus* Bleeker from Siam, Indo-China and Malaya is only a subgenus of *Mystus*. *M. seenghala* (Sykes), *M. aor* (Hamilton) and *M. leucophasis* (Blyth) are separable under a new subgenus *Osteobagrus* Jayaram (1955, pp. 529 and 547). Whether any such subgeneric complexes are present in the Malayan, East Indian, Chinese, North-East Asian species is being investigated.

17. **Horabagrus** Jayaram—*Bull. Nat. Inst. Sci. India*, no. 7, 1955, 261 (generic type by original designation and monotypy *Pseudobagrus brachysoma* Günther).

The circumstances under which this genus was proposed have been discussed in an earlier article (Jayaram, 1952). This is a monotypic genus found in Peninsular India along the Malabar coast. Its affinities are with the Palaearectic genus *Pelteobagrus* from which it differs in having low set eyes visible when viewed from below, short barbels, and a long anal fin with 27-28 rays. It is also allied to *Crano-glanis* Peters.

SIAM, MALAYA AND EAST INDIAN GENERA

18. **Heterobagrus** Bleeker—*Versl. Med. Akad. Wet. Amsterdam*, XVI, 1864, 354 (generic type by original designation and monotypy *H. bocourti* Bleeker).

This monotypic genus is only subgenerically different from *Mystus* Scopoli as stated earlier. Examination of material of *H. bocourti* Bleeker justifies that *Pra-jadhipokia rex* Fowler based on differences in gill raker counts is within the range of specific variation of *Heterobagrus bocourti* and that it is synonymous with *P. rex*. This genus is probably evolved from the widely distributed and generalized genus *Mystus* and has diverged from it only subgenerically. An osteological comparison with *Mystus* is being undertaken.

19. **Bagroides** Bleeker—*Nat. Tijdschr. Ned. Ind.*, II, 1851, 204 (generic type by original designation *Bagroides melapterus* Bleeker).

All the three known species of this genus have been examined. *Leiocassis vaillanti* Popta is synonymous with *Bagroides macracanthus* Bleeker. The genus is allied to *Heterobagrus* Bleeker.

20. **Bagrichthys** Bleeker—*Ichth. Arch. Ind. Prodr.*, I, Siluri, 1858, 130 (generic type by original designation and monotypy *Bagrus hypselopterus* Bleeker).

This is a monotypic genus related to *Synodontis* Cuvier found in Africa. Both genera resemble each other in having the mandibularly barbels branched, body compressed, adipose fin large, long, labial teeth enlarged and movable, and caudal fin filamentous (only in some species of *Synodontis*). *Bagrichthys* differs from *Synodontis* in having a dentigerous palate, four pairs of barbels (*versus* three pairs), gill membranes free from the isthmus and in not possessing a cephalonuchal shield. It appears evident that *Synodontis* has evolved from *Bagrichthys*-like fishes.

JAPANESE AND CHINESE GENERA

21. **Pseudobagrus** Bleeker—*Acta. Soc. Sc. Indo-Neerl.*, VIII, 1860, 87 (generic type by original designation *Bagrus aurantiacus* Temminck and Schlegel).
22. **Pelteobagrus** Bleeker—*Ned. Tijdschr. Dierk.*, II, 1865, 9 (generic type by original designation *Silurus calvarius* Basilewsky).
23. **Leiocassis** Bleeker—*Ichth. Archipel. Ind. Prodr.*, I, Siluri, 1858, 139 (generic type by original designation *Bagrus poecilopterus* Valenciennes).

These three genera are involved in a complex group, separation of them on external features being almost impossible. Nichols (1943) stated that recognition of these genera is only a matter of convenience. Günther (1873), Tchang and Shih (1934), Kreyenberg and Pappenheim (1909) and others felt this complexity and considered these genera either as synonyms or as subgenera of *Mystus* Scopoli. *Leiocassis* possesses a well-developed post-temporal plate which is in varying degree of development in the other genera, *Pseudobagrus* having the most ill-developed one. Regan (1911, p. 561) separated *Pseudobagrus* from *Leiocassis* on the modification of the pterygoid bones being laminar and loosely connected in the former and united suturally in the latter, and on the modification of the parapophyses of the IVth vertebra. It is obvious that generic fixation based on such features, although very sound, involves considerable damage to the specimens; in many instances they being unique representatives for the concerned museum. A combination of the caudal fin shape, being forked or otherwise; shape, size and position of the eyes; cranial roof being covered or uncovered with skin and the count of the anal fin rays appear to be of diagnostic value. On this basis, many out of the 48 species of *Leiocassis* so far known, have been reassigned either to *Pelteobagrus*, *Pseudobagrus* or *Mystus*.

The following are a few other noteworthy points:—

1. *Hemibagrus taphrophilus* Sauvage is likely to be a synonym of *Pseudobagrus medianalis* (Regan).
2. A neotype of *Bagrus vachellii* Richardson has been selected.
3. The generic status of *L. hirsutus* Herre appears to be doubtful in view of its peculiar features.

4. *L. torosilabris* Sauvage is not a *Bagrichthys* as was considered by Regan (1913) and others, but is synonymous with *L. crassilabris* Günther.
5. The generic type of *Leiocassis* is undoubtedly *Bagrus poecilopterus* Valenciennes contrary to other views of considering *Bagrus microgogon* Bleeker as the type.
6. The generic spelling of *Leiocassis* was unnecessarily emended to *Liocassis* by Günther (1864) in the absence of any inadvertent error or nomenclatural preoccupation.
7. *Leiocassis longirostris* Günther and *L. dumerili* Bleeker are homonyms and *longirostris* has precedence over *dumerili* being the one to be published two months earlier.
8. *L. doriae* Regan, *L. merabensis* Regan and *L. hosii* Regan, all known from Borneo by their respective type-specimens and with their range of morphometric characters over-lapping appear to be all probably subspecifically related to the widely distributed *L. baramensis* Regan.

SUMMARY

After reviewing the nomenclatural and classificatory changes undergone by the fishes of the family Bagridae, a list of the now valid fossil and living genera is given. The genera inhabiting the African, Indian, Siam, Malaya and East Indian and the Japanese and Chinese areas are grouped together phylogenetically where necessary, and the systematic problems associated with each genus are discussed. Work so far completed on each of the genera is also given.

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NOTES ON FOUR DEFORMED SPECIMENS OF THE INDIAN CARP, *LABEO ROHITA* (HAMILTON)

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INTRODUCTION

The occurrence of malformations in fishes is not uncommon and when present it is usually apparent even to a casual observer. Gemmill (1912) reviewed work on the abnormalities of bony fishes but information then available on the carp group was very scanty. Only one record of a hump-backed *Catla* has so far been made from India by Hora (1942), which has been subsequently worked out by Law (1944).

This paper deals with four fishes, which in spite of their deformities possess the typical specific characters of *Labeo rohita*. These fishes were collected from the river Jumna and its neighbouring ponds during the months of October to December, 1953, within Delhi State. Specimens are adults and measure 13.7 cm. to 30.8 cm. in standard length. It is of interest to note that all the major external deformities, as noted in the specimens examined, are limited to the dorsal and lateral sides of the body. In two specimens there is coalescence of vertebral centra resulting in bends, while in the other two, bends are without any coalescence of the vertebral centra.

To study deformities, comparison has been made with the normal fish of the same head-length, this being the least affected part in specimens. The deformities have been confirmed with X-ray examinations and dissections of the vertebral columns.

The vertebral column of a normal *L. rohita* has 37 vertebrae, of which the first four unite as in all other physostomous fishes to form a compound vertebra. Of the 37 vertebrae, 21 belong to the trunk region and are provided with 17 pairs of ribs, beginning from the fifth vertebra while the remaining belong to the caudal region and have haemal spines (Sarbahi, 1932).

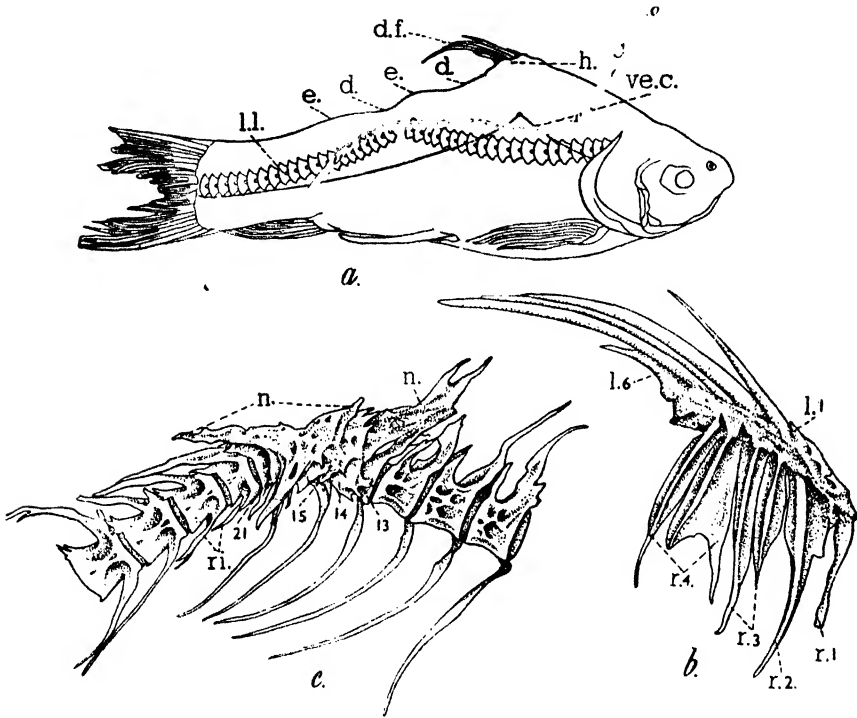
I. COALESCENCE OF VERTEBRAL CENTRA

Specimen No. 1

Deformities (Text-fig. 1. *a.*) are observed externally from the dorsal fin to the beginning of caudal peduncle. A cone-like hump is seen on the dorsal side and the incomplete dorsal fin is present on it. This hump is followed behind by two dorsal depressions and two elevations. Lateral bulgings or swellings are at the level of the first dorsal elevation.

The depth of the body at the hump is greater than that of the normal fish. The dorsal fin which has considerably shifted forward from its normal position is situated above the middle of the pectoral fin and has only six anterior fin rays, the posterior portion being completely absent. There is neither a scar nor any sign of injury at the place of missing dorsal rays and this area is completely covered by the scales. The other fins also show somewhat displaced positions in relation to the other parts of the body and have normal number of fin rays.

Lateral line on both the sides is interrupted, that of the right side is discontinuous after the 19th scale and then continues again from 21st scale; and of the left side shows a break after the 14th scale and then continues from 23rd scale.



TEXT-FIG. 1.

- Text-fig. 1. *a.* Specimen No. 1 showing the hump, depressions, elevations, deformed dorsal fin, break in the lateral line and the position of the deformed vertebral column. (Right side \times c.a. $\frac{1}{2}$).
 „ „ *b.* Radials and lepidotrichia of the deformed dorsal fin of specimen No. 1. ($\times 1$).
 „ „ *c.* Part of the vertebral column of specimen No. 1 showing the coalescence. ($\times 1$).

In the trunk region (Text-fig. 1. *c.*) the first coalescence of centra is observed in 13th and 14th vertebrae. The two neural spines are fused just above the neural arches while their distal ends are free. Only one pair of pleural ribs is observed in place of two pairs. The second coalescence of centra is noted in 15th to 21st vertebrae and this portion is irregularly contorted laterally. The neural spines of these vertebrae are in form of small irregular projections directed in anterior and posterior directions. A groove on the dorsal side of deformed vertebrae represents their neural canal portion, while small ventral projections indicate the ribs. The neural spines of the trunk vertebrae are directed forward instead of having the usual backward direction. The vertebral column forms an obtuse angle at the hump part.

The dorsal fin (Text-fig. 1. *b.*) has only six lepidotrichia instead of the typical 15 to 16. Four radials are present of which the first one is smaller and more irregular than the remaining three. The first two unbranched lepidotrichia are supported on the second radial, the third and fourth on the third, and the fifth on fourth radials respectively. The sixth lepidotrichia is without any radial support.

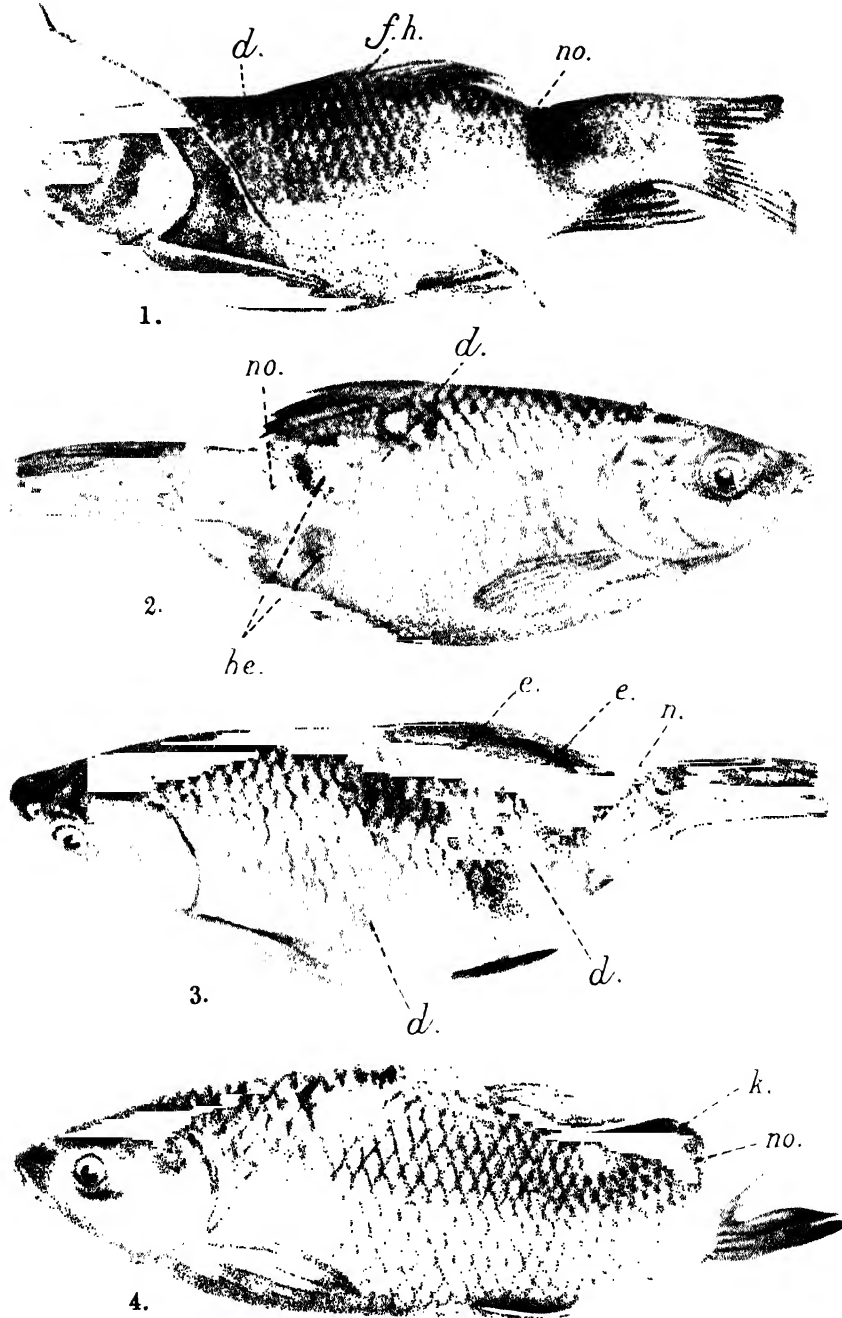


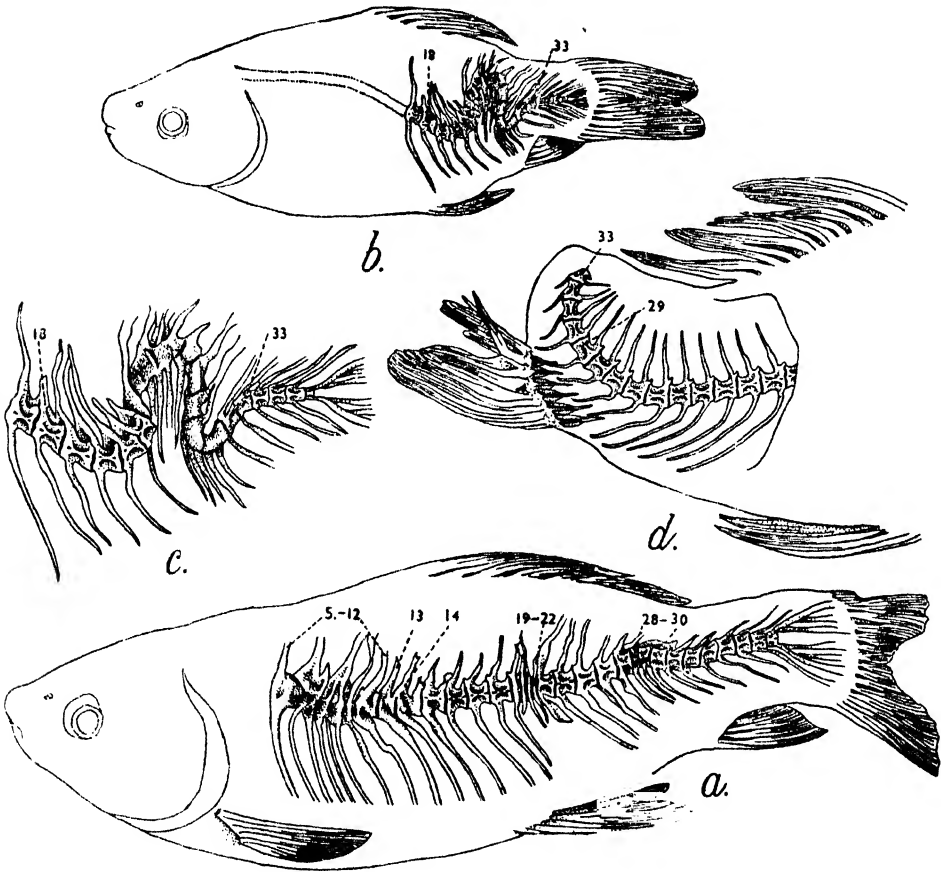
Plate Figs. 1-4.

Photographs of the deformed specimens of *Labo rohita* (Ham.).

Fig. 1. Specimen No. 2, Left side; Figs. 2, 3. Specimen No. 3. Right and left side respectively; Fig. 4. Specimen No. 4. Left side

Specimen No. 2

The elevations and depressions in this specimen (Plate XIV, Fig. 1.) are slightly different in position as compared to the first. There is only one shallow depression behind the head region after which is a hump-like flat bulge or elevation. A distinct notch at the beginning of the caudal peduncle on both the sides is present. The entire caudal peduncle, immediately behind the notch, is bent from left to the right side. The position of various fins and the number of rays are normal. The lateral line is, however, interrupted only on the right side with 27 scales in the first part and 11 scales in the second part.



TEXT-FIG. 2.

- Text-fig. 2. *a.* Vertebral column of the specimen No. 2 showing the coalesced and the non-coalesced portions. ($\times \frac{1}{2}$).
b. Non-coalesced portion of the vertebral column with bends in specimen No. 3. ($\times \frac{1}{2}$).
c. Magnified view of the vertebral column in specimen No. 3.
d. Non-coalesced portion of the vertebral column with upward bend in specimen No. 4. ($\times \frac{1}{2}$).

The first deformity (Text-fig. 2. *a.*) is from 5th to 14th vertebrae where the centra of 5th to 12th vertebrae are coalesced. The 13th and 14th vertebrae though not,

coalesced are deformed because of curvature. The neural spines of 8th to 10th vertebrae are fused. The second deformity is due to coalesced centra of 19th to 22nd vertebrae. The neural spines of 20th and 21st and also of 22nd and 23rd vertebrae are fused while the parapophyses are normal. The third deformity is on account of coalescence of centra of 28th to 30th vertebrae. The neural spines of these vertebrae are fused but the usual number of haemal spines are present. The centra of the vertebrae are more or less compressed and short on the left and ventral sides, while they are broad on the right and dorsal sides and hence the curvature in this portion.

II. NO COALESCENCE OF VERTEBRAL CENTRA

Specimen No. 3

The deformities in this specimen commence (Plate XIV, Figs. 2, 3.) from the beginning of the dorsal fin and extend up to the root of caudal fin. The axis of the fish is sharply bent towards the right side near the caudal peduncle which is short and narrow in comparison with the size of the body.

The depressions and bulgings or elevations are observed on the lateral sides. On the right side there is a single depression and a single bulge with two distinct heads, while the left has two depressions and two elevations. A transverse notch is observed at the beginning of the caudal peduncle on both the sides. The noticeable features are the backward displacement of the dorsal, pectoral and pelvic fins, shortness of the caudal peduncle and increase in the dorso-ventral diameter of the body. Fins have the usual number of rays.

Lateral line on the right side is not distinctly seen throughout and only 18 scales could be counted at different places. On the same side the scales near the depression and notch are all crowded together. On the left side the lateral line has a break after 21st scale and continues in the second part with 14 scales.

The vertebral column (Text-fig. 2. *b* and *c.*) of this fish has short sharp bends from 18th to 33rd vertebrae, where the centra are complete, independent but abnormal. Owing to the bends, associated structures of all deformed vertebrae show change of slope and directions. The bends of the deformed region are towards lateral, upward and downward sides.

Specimen No. 4

This specimen (Plate XIV, Fig. 4.) differs markedly from the other three in having no depressions and elevations, and in the absence of a portion of the caudal peduncle and the caudal fin. The remaining portion of the caudal peduncle forms a blunt truncated stump which is bent upward. On the left side, above the lateral line, an oblique notch is present near the tip of the caudal peduncle. The number of rays in the existing fins is normal. The lateral line on both the sides is continuous and has only 35 scales instead of 40-42 scales.

There are only 33 vertebrae instead of normal 37, posterior four caudal vertebrae being absent (Text-fig. 2. *d.*). The posterior part of the vertebral column is bent upwards and centra of the vertebrae constituting this bend, have shorter dorsal surfaces than the ventral. The last five, 29th to 33rd, caudal vertebrae are slightly bent from the right to the left and have their neural spines curved. The 29th to 32nd vertebrae on the left side have deep depressions on their centra. The last 33rd vertebra has an open groove which marks the position of the arch. The centrum of the last vertebra forms a nodule-like structure.

The differences observed in measurements, scale counts and fin rays in the abnormal specimens as compared to the normal specimens of the same head-lengths, are given in the table.

Differences in measurements (in centimetres), fin rays and scale counts of abnormal and normal specimens of Labeo rohita of the same head-length.

	Specimen No. 1		Specimen No. 2		Specimen No. 3		Specimen No. 4	
	Head-length 9.0 cm.		Head-length 7.2 cm.		Head-length 4.5 cm.		Head-length 6.1 cm.	
	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal
Total length ..	38.1	42.0	31.8	35.2	17.7	19.9	23.5	28.8
Standard length ..	30.8	33.8	25.0	28	13.7	15.6	19.0	23.2
Height of the body at the commencement of the dorsal ..	11.1	11.3			5.9	4.6	7.0	7.2
Height of the body at the middle of the dorsal ..	13.3	10.3	7.4	7.5	5.3	4.0	6.0	6.3
Height of the body at the end of the dorsal ..	11.2	7.9	6.5	6.6	4.0	3.4	4.7	5.5
Distance between the tip of the snout and commencement of dorsal ..	13.9	16.0	12.2	13.5				
Distance between the tip of the snout and commencement of pelvic ..	16.0	20.0	14.3	15.8	L 9.0 R 8.7	8.5		
Distance between the tip of the snout and commencement of anal ..	22.1	28.3	20.4	22.5	L 11.8 R 11.6	13.8		
Distance between the anal and pelvic ..	6.8	10.2	7.3	9.1	L 3.6 R 3.4	4.5	7.5	6.2
Distance between the pectoral and pelvic ..	8.6	11.9	7.7	8.5	L 5.0 R 4.0	4.2		
Length of the base of the dorsal ..	1.9	7.5	5.1	5.7				
Length of the caudal peduncle ..			L 3.8 R 3.0	4.9	1.8	2.4		3.7
L. 1 of left side ..	33	42	40	42	35	40	36	40
L. 1 of right side ..	40	42	38	42	18	40	36	40
L. tr. ..	6½, 9	7½, 6½	7½, 6	7½, 6½	L 7½, 6½ R 7½, 5½	7½, 6½		

L = Left Side; R = Right Side

DISCUSSION

The deformities observed in fishes in the past and their probable causes have been explained by Gemmill and others. Our observations on malformations in four fishes endorse with and differ from the view of many, but diversity of opinion still exists on the percentage and the cause of such deformations. Synostosis of the vertebral centra and the occurrence of simple bends have been mostly observed by earlier reporters. The feature of synostosis of vertebral centra in two adjacent groups in the first specimen, and in three separate groups in the second specimen, endorses the observations by Gemmill, and Law.

In almost all the deformed specimens the number of neural spines and haemal spines and the pleural ribs are not normal. All the features are different from those recorded by Cobbold (1835), Dyce (1860), Smith (1864-65), Howes (1894), and Ritchie (1908).

The deformities of the vertebral column in the first specimen correspond with the external conical hump, while depressions and elevations have no corresponding vertebral deformities.

The depression in the second specimen corresponds to the first deformed portion of the vertebral column. The portion of the vertebral column corresponding to the elevation with a flat surface does not show any deformity except in the middle region only in a few vertebrae. Deflected region at the beginning of notch corresponds to third group of deformed vertebrae.

The deformities in the first specimen seem to be due to mechanical injury at an early stage caused by some violence followed by irregular calcification. The first and the third deformities in the second specimen are similar to those observed in the first specimen, but the second deformity in the second specimen is due to compression at the time of the formation of vertebral centra as evidenced by their distinct centra which are telescoped into one another. Continuous ossification must have followed the compression resulting in the formation of a compound bone. The congenital factors may be the cause of such a deformity. Law, and Ritchie have observed similar deformity in *Catla* and trout respectively.

Hyrtl's statement (1862) that 'synostosis takes place more frequently in the tail than in the trunk of the fish' is not in conformity with the above observations where synostosis in the vertebral centra has occurred more in the trunk portion than in the tail region. Law, however, found synostosis in the entire vertebral column.

The complex curvatures of the vertebral column in the third specimen corresponds with the latter part of external deformity due to which the whole of the caudal peduncle is considerably shortened.

The curvatures in this may be due to mechanical disturbances or unfavourable influences in the affected part of the body during the period of development. Howes in a sole and Storow (1909) in a cod fish observed sharp sinuosities which were not confined to a particular part of the vertebral column similar to those described by us.

The upward bend of the vertebral column of the fourth specimen corresponds to the external feature of the body, and this deformity is due to some injury. This is due to the premature calcification in the dorsal portion of all the centra of the caudal vertebrae.

The presence of a short dorsal fin with only a few rays in specimen No. 1 is a consequential change and shows that deformity of the vertebral column took place at an early stage which affected the fin also. The caudal fin in specimen No. 4 seems to have been bitten off by a predatory fish and the bitten portion healed up and regenerated as deformed.

Displaced fins from the normal position, as we have described here, were also recorded by Law. Gemmill and others while discussing the abnormalities of fins made no reference to the external structure of these defective fins. Law has

stated that 'there is considerable derangement in the bones supporting the caudal fin' but the detailed description is wanting. The causes mentioned by Gemmill about the absence and defective growth of fin are also applicable in the present specimens. The first case is due to defective development of fins and the fourth to 'injury or removal followed by healing'.

Law observed that abnormalities in fishes are very rare but McHugh and Barraclough (1951) have mentioned that 'fusions and deformities involving a limited number of vertebrae are not uncommon in natural populations of fishes'. In Atlantic and Pacific herring such anomalies are found in as many as 14 per cent of the individual in a sample (Ford and Bull, 1926; McHugh, 1942). Malformations are seen in many species of teleost fishes as recorded by Wunder (1939), Ninni (1942), O'Donnell (1945), Oselladore (1949-50), and they have reviewed the earlier work of this nature. Law states that it is too premature to make any comment whether environment plays any part or not. Kirpichnikov (1945) states that 'the body form and rate of growth of the carp are known to be extremely sensitive to the effects of environment'. But as the present specimens are collected from different sources of water, the nature of which is not known to us, we cannot comment much on this view.

ACKNOWLEDGEMENTS

We are indebted to Dr. M. L. Bhatia for guidance. We are grateful to Dr. S. L. Hora for kindly helping us with valuable references, suggestions and also for reading through the manuscript. Thanks are also due to Mr. J. N. Rudra for assisting us in the preparation of manuscript, and also to the artist, Mr. Kasturi Lal Bhalla, for making figures.

SUMMARY

The record on malformation in fishes is not rare and this communication deals with four deformed specimens of *Labeo rohita* (Hamilton). External features, vertebral column and elements of the fins are studied in these carps.

The deformities of vertebral column are grouped into two categories: (i) no coalescence of vertebral centra and (ii) coalescence of vertebral centra. The deformities in the first specimen, as well as the first and the third deformities in the second specimen are probably due to mechanical injury at an early stage caused by some violence followed by irregular calcification. The second deformity in the second specimen is due to compression at the time of formation of vertebral centra and this may be attributed to congenital factors. In the third specimen curvatures in the vertebral column may be due to mechanical disturbances or unfavourable influences during the period of development, and in the fourth, the deformity is clearly due to some injury.

The causes of deformed fins in the specimens are either due to defective development or the injury or removal followed by healing.

The statistical data given by different workers indicate that deformities of vertebral column in fishes are not uncommon in nature. The role played by environment in bringing about such abnormalities is still a controversial issue.

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EXPLANATION OF LETTERING IN TEXT-FIGURES AND PLATE XIV

d.—depression; *d.f.*—dorsal fin; *e.*—elevation; *f.h.*—flat hump; *h.*—hump; *he.*—head; *k.*—knob; *l.l.*—first lepidotrichia; *l.6.*—sixth lepidotrichia; *l.l.*—lateral line; *n.*—neural spine; *no.*—notch; *r.1.*, *r.2.*, *r.3.*, *r.4.* number of radials; *ri.*—ribs; *ve.c.*—vertebral column. 13, 14, 15, 21, 18, 33, 5-12, 19-22, 28-30, 29 number of vertebrae.

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THE STIPULES, PULVINUS AND STIPELS OF *DOLICHOS* *LABLAB* L. AND THEIR VASCULAR SUPPLY

by ASOK KAR, Botanical Laboratory, Presidency College, Calcutta

(Communicated by G. P. Majumdar, F.N.I.)

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INTRODUCTION

Dolichos lablab is a herbaceous, prostrate or twining, plant cultivated in Bengal and elsewhere for its edible pods. Leaves are pinnately trifoliate; stipules are small, subpersistent, *cauline* (Parkin, 1948); stipels subulate, one to each of the lateral leaflets, but a pair to the terminal one; leaves and leaflets are provided with pulvini. The pulvinus of the leaf runs into the rachis which is long, grooved on the upper surface flanked by two prominent ridges which disappear at the divergence of the first pair of leaflets, but appear again on the other side and run up to the base of the terminal leaflet (Fig. 1).

VASCULAR SUPPLY TO THE STIPULES

Since the time of Colomb (1887) the origin and nature of vascular supply to the stipules has been thoroughly studied, and it is now accepted as a morphological dictum that the stipular traces are derived exclusively from the laterals of the trace of the leaf to which they belong (Sinnott and Bailey, 1914; Dormer, 1944; Mitra, 1945, 1948, 1949a, 1949b, 1950a, 1950b, 1950c). Mitra and Mitra and Majumdar (1952) have shown that all types of stipules (*cauline* and *adnate*, Parkin, 1948) are leaf-base outgrowths.

In *D. lablab* the node is pentalacunar and five bundles form the leaf trace, one median (*M*) and four laterals (*L*₁, *L*'₁, *L*₂, *L*'₂), two in each wing (Fig. 2). The laterals leave the axial cylinder before the median. On their way to join the median at the base of the pulvinus the laterals jointly give out branches in the foliar foundation (leaf-base) and each stipule receives about five feeble branches which form its trace (Fig. 3). The stipules do not develop to a great extent but they are wide at the base and subpersistent.

VASCULAR SUPPLY TO THE PULVINUS

The four lateral bundles (*L*₁, *L*'₁, *L*₂, *L*'₂) unite with one another and with the median to form a closed ring of vascular bundles in the pulvinus (Fig. 4). Sinnott and Bailey (1915) in tracing the factors involved in foliar evolution state that 'the primitive leaf with its three (or more) traces, widely separate in origin passing directly from node to lamina, was thus constricted at its base and its three (or more) bundles-forced close together in the petiole' (pp. 13, 14). This feature of vascular system is characteristic of the petiole and not of the leaf-base. The pulvinus, therefore, on anatomical evidence cannot be regarded as the base of the leaf as has been suggested by Green (1897), Vines (1910), Lawson and Sahnj (1949) and others.

Mitra (1950c) and Mitra and Majumdar (1952) have elaborated the above statement of Sinnott and Bailey (1915) and have shown that anatomically the

leaf-base and the petiole can be distinguished one from the other by the behaviour of the leaf trace bundles while passing through them to the lamina. In the *leaf-base* the laterals of the trilacunar and multilacunar nodes after leaving the axial ring run parallel for some distance and then bend horizontally or obliquely towards the median and they unite to form a closed ring, or an open arc, or a ring of vascular bundles at the base of the petiole and remain so up to its top. In *D. lablab* after a short parallel course they run horizontally in the leaf-base and unite with the median to form a closed ring in the pulvinus (Figs. 3 and 4).

Rachis is the 'back-bone' of the leaf and is defined 'as the prolongation of the petiole in a pinnate leaf forming the principal axis from which leaflets are given off' (Heinig, 1899). In *D. lablab* the pulvinus prolongs into the rachis. If the pulvinus is the leaf-base as held by the European authors, then the leaf of this plant has no petiole which seems untenable on the evidence presented here.

Stipels.—Stipels are defined as the 'stipules of a leaflet', but with a difference. Asa Gray (1879) describes stipels as 'small and slender and unlike stipules they are single to each leaflet except to the terminal one which has a pair' (p. 106; cf. also Heinig, 1899; Green, 1897; Vines, 1910; Lawrence, 1951 and others). None of them, however, appears to have studied the true morphology of the stipel except Goebel (1905).

Goebel (II, pp. 379–381) distinguished two different types of stipels: one is independent in origin, whereas the other type is a reduced pinnule. The first type is exemplified in a few species of *Thalictrum* (his Fig. 254). Such stipels occur in pairs to each leaflet, and they are comparable to a pair of stipules at the base of a stipulate leaf.

Stipels, which Goebel regarded as reduced pinnules, occur in *Phaseolus*, *Robinia*, *Desmodium* and other Leguminosae (*D. lablab* is one of them) and are found at the base of their leaflets. He writes: 'They appear usually in the form of small teeth and occasionally they are developed as leaves, e.g. upon sucker shoots in *Robinia*.' But Goebel himself was not very sure of his interpretation. From their position on the rachis just below the pair of lateral and the terminal leaflets, the stipels may very easily be mistaken for a pair of reduced pinnules (Fig. 1).

In *D. lablab* stipels occur, as I have already pointed out, on the ventral surface of the rachis, one to each of the first pair of leaflets, and a pair at the base of the terminal leaflet. How are we to explain this difference in the distribution of the stipels in two positions on the rachis? Vascular supply to these organs give the clue for an answer.

VASCULAR SUPPLY TO THE STIPELS OF THE LATERAL PAIR OF LEAFLETS

Fig. 4 shows the nature of the leaf trace bundles immediately below the subapical region of the pulvinus. Just at the top of it where the pulvinus prolongs into the rachis the closed ring of bundles opens a little on its ventral side and an open arc is formed (Fig. 5). From each of the two ends of this open arc, i.e. from the distal ends of L_2 , L'_2 , a branch (rb , rb') is sent out to stimulate the growth and formation of the two ridges (pulvinus is cylindrical) in which they enter and a groove is formed between them (Figs. 6 and 7). In the rachis the five trace bundles become prominent again with small branches interconnecting them (Fig. 7). This arrangement continues up to the point of the divergence of the stipels at the base of the lateral pair of leaflets. Stipels appear directly on the rachis slightly below the articulation of the leaflets to which they belong (Fig. 1).

Branches from the laterals, L_2 and L'_2 , and from the ridge bundles (rb and rb') on the two sides of the rachis are sent respectively to the two stipels to form their traces (Fig. 8). Each stipel of a leaflet thus receives, like the stipule at the base of a leaf, its vascular supply from the branches of the lateral L_2 or L'_2 , and the leaflet

to which each stipel belongs, gets either L_2 or L'_2 but not both (see below), whereas the leaf with a pair of stipules receives both or all the laterals.

The stipules are, therefore, stipules to the leaflets and their position on the rachis and vascular supply explain the presence of only one stipel to each leaflet.

VASCULAR SUPPLY TO THE PAIR OF LEAFLETS

A little higher up the rachis the first pair of leaflets diverge. The laterals L_2 or L'_2 , the ridge bundles rb or rb' and a branch from L_1 or L'_1 enter the base of each leaflet on the corresponding side of the rachis, leaving only the median (M) and the laterals (L_1 and L'_1) to form the rachis bundle (Fig. 9).

BEHAVIOUR OF THE RACHIS BUNDLE BEYOND THE PAIR OF LEAFLETS

The rachis bundle, now constituted of only three original leaf trace bundles, proceeds to the terminal leaflet. Soon after passing the bases of the lateral leaflets L_1 and L'_1 send out a branch each to reconstitute the ridges and their bundles (Figs. 10 and 11). At the base of the terminal leaflet they (L_1 and L'_1) give out branches again, and each branch with the corresponding ridge bundle goes to constitute the vascular system of the stipels, now a pair at the base of the terminal leaflet (Fig. 12). In the pulvinus they unite with the median to form a closed ring of bundles, the characteristic feature of the petiole (Fig. 13). On entering the base of the terminal leaflet they resolve into five main branches which form the five ribs of the palmi-veined leaflet (Fig. 14).

DISCUSSION

The leaf and the leaflets: lateral and terminal.—Two or three very interesting features come out of these observations: Vascular supply to the leaf and the leaflets—lateral and terminal—does not follow the same pattern. The main leaf gets from the axial cylinder five bundles, one median and four laterals. Each lateral leaflet receives only one of these laterals (L_2 or L'_2), the ridge bundle (rb or rb' , which is also a branch of the lateral L_2 or L'_2) and a branch of the lateral L_1 or L'_1 . Altogether each leaflet receives three bundles with L_2 or L'_2 forming the median. On entering the blade they resolve into five main nerves of the palmi-veined blade. The terminal leaflet also receives three trace bundles, M , L_1 and L'_1 , which on entering the blade resolve into the five main veins of the palmate leaflet, the median forming the mid-vein. On the basis of the vascular supply, therefore, the lateral leaflets cannot be regarded as equivalent to a simple leaf, but it should be regarded as equivalent to its lobe or a tooth which is equally fed by a lateral trace bundle or its branch.

The stipules and the stipel.—The stipules as usual occur in pairs at the base of the leaf. Each stipule gets its vascular supplies from the laterals of the leaf trace while on their way to the base of the petiole (pulvinus). This supports the findings of Colomb and later authors. The stipel to the lateral leaflet is one. Like a stipule it gets its vascular supplies from the second lateral on the corresponding side of the rachis, the same lateral which goes to supply the leaflet to which the stipel belongs. The absence of the other stipel to this leaflet (on the analogy of stipules at the base of a leaf, there should have been two) is easily explained because the other lateral is on the opposite side of the rachis and it forms the trace of the leaflet on this side. But in the case of the terminal leaflet, both the laterals before entering the base of the leaflet give out branches to the two stipels just like what happens in the case of a stipulate leaf with a three-bundle trace. The rachis is the midrib which is a continuation of the petiolar region of the leaf. The *pulvini* of the main leaf and of the leaflets on anatomical evidence are really the petiole and petiolules and not the leaf-base modified as held by the European authors.

The morphological status of the lateral leaflets.—Are they equivalent to simple leaves? Their vascular supplies do not support their simple leaf nature. The leaf of *D. lablab* receives five bundles from the nodal ring, i.e. they cause five gaps in the axial cylinder. Each leaflet, on the other hand, receives the distal lateral bundle and a branch from the first lateral. The leaflet, therefore, cannot be regarded as equivalent to a simple leaf, but is homologous with a lobe or a tooth of the same.

SUMMARY

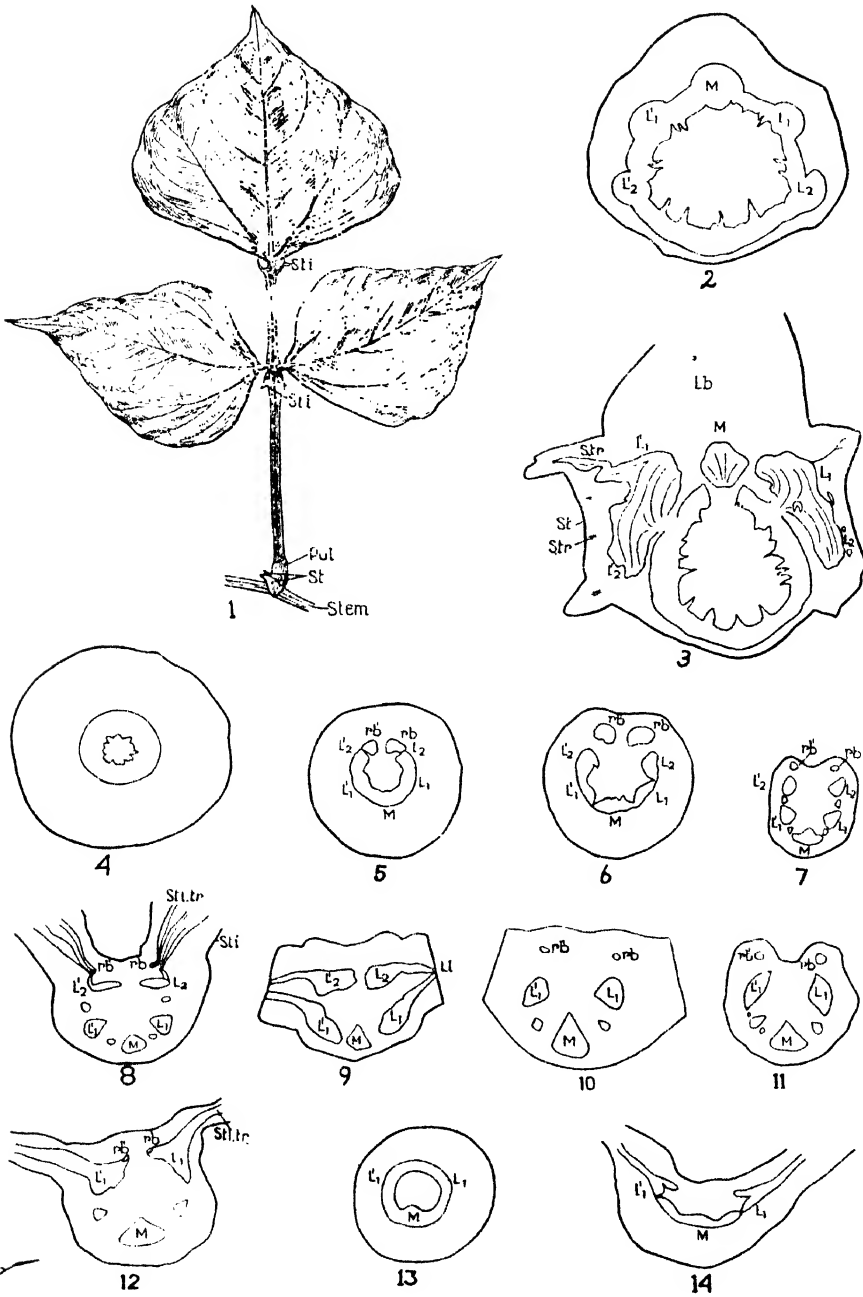
1. The node is pentalacunar. Two laterals from each side and the median leave for the leaf, causing as many gaps in the nodal ring.
2. Stipules are leaf-base outgrowths, and each of them receives branches from both the laterals on each side of the median.
3. Pulvinus is the petiole, and not the leaf-base modified to aid movement of the leaf.
4. Stipels are stipules of the leaflets as they receive branches from the same laterals which form their traces.
5. The presence of a single stipel to each lateral leaflet and a pair to the terminal leaflet is explained on anatomical evidence.
6. The lateral leaflets on anatomical grounds should be regarded as parts of the blade equivalent to its lobes or teeth fed equally by laterals or their branches.

ACKNOWLEDGEMENT

I express my gratefulness to Prof. G. P. Majumdar, under whose guidance the investigation was carried out.

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EXPLANATION OF TEXT-FIGS. 1-14

All figures drawn under a projection apparatus under magnification noted against each figure

- FIG. 1. A leaf of *D. lablab* showing the distribution of the stipules, stipels and leaflets. $\times \frac{1}{2}$.
 „ 2. T.S. through the base of the node showing five leaf trace bundles prior to their departure for the leaf. $\times 8.5$.
 „ 3. T.S. through the level of insertion of the leaf showing the union of the laterals and their movement towards the median, and the origin of the stipular traces from them. $\times 8.5$.
 „ 4. T.S. of the pulvinus showing the union of the median and the laterals to form a closed ring of bundles. The median and the laterals have lost their identity in forming the ring. $\times 8.5$.
 „ 5. T.S. through the junction of the pulvinus and the rachis. The closed ring has opened on the ventral side and two ridge bundles are cut off from the distal ends of L_2 and L'_2 . $\times 6$.
 „ 6. T.S. through the base of the rachis showing movement of the ridge bundles and the formation of the ridges. The median and the laterals can now be distinguished. $\times 6$.
 „ 7. T.S. of the rachis showing the ridges and the groove on its ventral surface and the five leaf trace bundles with their branches. $\times 6.5$.
 „ 8. T.S. through the bases of the stipels showing their vascular supplies from the laterals and the ridge bundles. $\times 6.5$.
 „ 9. T.S. through the base of the pair of leaflets showing their vascular supplies from the laterals, the median not taking part. $\times 6.5$.
 FIGS. 10 and 11. T.S. of the rachis beyond the pair of leaflets showing the beginning and re-appearance of the ridges and their bundles. $\times 6.5$.
 FIG. 12. T.S. through the bases of the stipels of the terminal leaflet showing their vascular supplies. $\times 6.5$.
 „ 13. T.S. of the pulvinus of the terminal leaflet showing the ring of vascular bundles formed by the union of M , L_1 , L'_1 . $\times 6.5$.
 „ 14. T.S. through the base of the blade of the terminal leaflet showing the midrib region and the distribution of the first pair of veins. $\times 6.5$.

Legend:—Lb—Leaf-base; St—Stipules; Pul—Pulvinus; Sti—Stipel; Ll—Leaflet; S.tr—Stipule trace; Sti.tr—Stipel trace; M—Median, and L_1 , L'_1 , L_2 , L'_2 —Laterals of the leaf trace; rb and rb' —ridge bundles.

THE GAMETOPHYTE OF *ALEURITOPTERIS GRISEA* (BLANFORD) COMB. NOV.

by G. PANIGRAHI, *Ravenshaw College, Cuttack.*

(Communicated by I. Banerji, F.N.I.)

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INTRODUCTION

The genus *Aleuritopteris* was originally founded by Fée (1852) on the type species, *Pteris farinosa* Forsk. Kaulfuss (1824) considered *Aleuritopteris* synonymous with *Cheilanthes* Sw. and published the new combination, *C. farinosa* (Forsk.) Kaulf. Blanford (1886), however, recognised *C. farinosa* as a species complex and published *C. grisea* Blanford from Simla as a species distinct from *C. farinosa* (Forsk.) Fée sensu stricto. Ching (1941) and Copeland (1947) have, on the other hand, maintained that *A. farinosa* (Forsk.) Fée be re-established on the authority of the 'Type method'. Ching (l.c.) treats *C. grisea* as a variety of *A. farinosa* (Forsk.) Fée. Available evidences, both morphological and cytological (Manton and Panigrahi, unpublished), shall be utilised to show that *A. farinosa* (Forsk.) Fée complex comprises at least three taxonomic species of which *A. grisea* (Blanford) comb. nov. is one. The present investigation which deals with the haploid gametophyte of *A. grisea* was incidental to the studies in cytotaxonomy of *A. farinosa* (Forsk.) Fée complex under the supervision of Professor I. Manton of Leeds (cf. Manton and Sledge, 1954), to whom I am indeed grateful.

MATERIAL AND METHODS

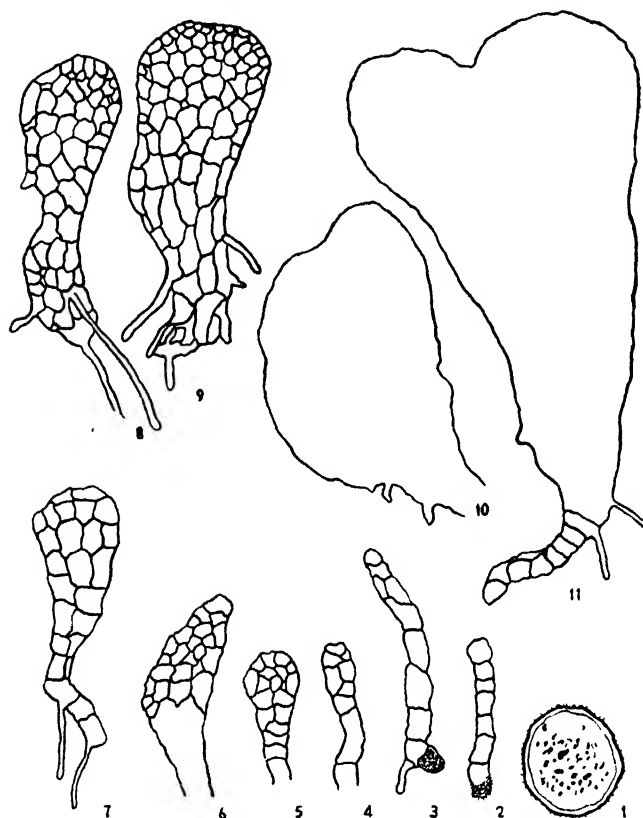
Spores of *A. grisea* were collected from plants which came from Ceylon and were kept in cultivation in one of the tropical fern houses in the Royal Botanical Garden, Kew.

Spores were sown in pots (2") containing a sterilised soil mixture made up of peat: loam: and silver sand in proportions of 3 : 2 : 1 and was sterilised in a steam steriliser at 180°F. for 20 minutes. The mixture was cooled immediately by spreading it out and was left for at least three days before using it in porous earthenware pots for sowing. The spores sown, the pot was soaked with water from below by standing it in a tray of water.

The account is based mostly on the study of fresh material, but for sex organs sections were cut from prothalli embedded in paraffin after fixation with half-strength Chromo-acetic-formalin (cf. Manton, 1950). Sections were cut at 10 μ and stained with Haidenhain's haematoxylin counterstained with Bismark Brown.

OBSERVATIONS

The spores of *A. grisea* are spherical, more or less smooth, brown with a thin exospore and average 45 μ in diameter (Fig. 1). Germination begins in 3-4 weeks time with the cracking of the exospore when a papilla emerges, which soon cuts off a filament of 6-8 cells (Figs. 2-3). The first rhizoid is formed from the basal cell of the filament (Fig. 3). The apical cell, at this stage, enlarges and cuts off cells by oblique cell walls (Figs. 4-5). In some cases division may initiate in cells one or two removed from the apical cells (Fig. 3), which, in its turn, develops a papillate



TEXT-FIG. 1.

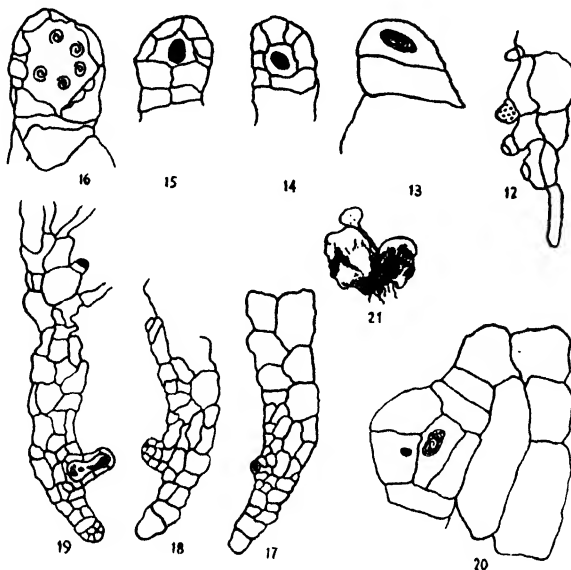
FIGS. 1-11. Fig. 1. Spore. $\times 350$. Figs. 2-3. Early stages of the filamentous thallus with a basal rhizoid; Figs. 4-5. Young plate stage; Figs. 6-10. Asymmetrical development of the prothallus, with apical cell of the filament pushed to a lateral position; Fig. 11. Cordate shaped prothallus, 5 months old with a filamentous base. Figs. 2-11 $\times 87$.

outgrowth (Fig. 6). Pronounced meristematic activity posterior to the apical cell results in asymmetrical development of the cell plate pushing the papillate apical cell to a lateral position (Fig. 8). The broadening of the filament into a plate is brought about by division of the cells from the apex. But the basal cells do not divide, so that even in large prothalli one can see a filamentous base (Fig. 11) except in old prothalli where only the basal cell remains undivided.

Cells in the plate towards the base and centre are much larger than the peripheral cells (Fig. 9), some of which at the central region differentiate into an apical meristem and cut off cells laterally resulting in a deep notch along the longitudinal axis of the plate (Fig. 10). Gradually, a cordate shaped symmetrical prothallus is formed with well developed wings of only one layer of cells (Fig. 11). The prothallus consists of 4-5 layers of cells at the sexually mature stage (Figs. 17-18), although a thick midrib is differentiated in very old prothalli prevented from fertilisation. The margin and surface of the prothallus is devoid of any emergence, such as hairs, setae or glands (Figs. 2-11 and 19).

The young rhizoids are colourless, but older ones are brownish. The basal cell of the filament bears only one rhizoid (Figs. 7-8), but these soon develop from the marginal cells of the gametophyte, particularly from its basal region (Figs. 8, 9 and

11). In very old prothalli, copious rhizoids, woolly in appearance develop from the midrib, sometimes on both the surfaces (Fig. 21).



TEXT-FIG. 2.

FIGS. 12-16. Antheridia median section. FIGS. 12-15. Early stages. FIG. 16. Mature antheridium with spirally coiled antherozoids. $\times 87$. FIGS. 17-18. Archegonium, stages in development. $\times 87$. FIG. 19. Mature archegonium. $\times 90$. FIG. 20. A magnified view of the developing archegonium. $\times 350$. FIG. 21. An old prothallus, woolly in appearance, with a young sporeling whose surfaces are covered with palish yellow ceraceous covering, one year old. $\times 2$.

ANTHERIDIUM

Antheridia first appear in prothalli, 3-4 weeks old and are never very abundant even on adult prothalli. There is usually zonation in the development of sex organs and the formation of antheridia is checked when archegonia are formed near the notch. But in prothalli, a year old, antheridial formation again takes place not only on the ventral surface but on the dorsal surface as well.

The antheridium initial arises as a protuberance from the superficial cell (Fig. 12) and is soon cut off by a transverse wall into a basal cell and an antheridial initial proper. The basal cell may divide again transversely to form the stalk of the antheridium (Figs. 13-15). The antheridium initial proper divides into an outer wall cell and an inner dome-shaped cell. The outer cell divides anticlinally into a layer of 5-8 cells (Figs. 14-16), of which one is cap cell. These constitute the antheridial wall. The inner dome-shaped cell divides several times mitotically to produce androcytes which transform into the ciliated antherozoids (Fig. 16). The antherozoids, when liberated by the dehiscence of the antheridium in contact with water, can be seen actively swimming about, under the microscope. These retain their motility up to half an hour after which these become inactive. The antherozoids, when fixed and stained, appear as coiled bands inside the antheridia (Fig. 16). The antheridia are globular or very slightly elongated.

ARCHEGONIUM

The archegonium develops from one of the superficial cells on the ventral side, usually 4-8 cells removed from the apical growing cell (Fig. 17). It divides

transversely into an outer primary neck cell and a larger inner cell (Fig. 20). The former as usual divides successively into 4 surface cells, from which differentiate the neck of 4 rows of cells, with 4-5 cells per row. The inner cell divides transversely into a ventral cell and a neck canal cell (Fig. 19). The nucleus of the latter divides again to form a binucleate neck canal cell. The ventral cell divides into a large egg and a small ventral canal cell (Fig. 19), both of which are surrounded by the cells of the venter formed from the surrounding prothallial cells. Thus, a fully developed archegonium is formed. Later, the ventral canal cell disorganise and are exuded out of the archegonium through the opening between the cover cells, thus establishing an open canal for the entrance of the ciliated antherozoids.

The first juvenile frond of the sporophyte is produced in 3-4 weeks from the date of fertilisation of the egg. The sporophyte develops greyish yellow ceraceous covering on both the surfaces of the frond and the stipe, which retards the rate of transpiration (Fig. 21).

DISCUSSION

Stokey (1951) has shown that the contribution of the gametophyte is of taxonomic significance and may be utilised for the classification of homosporous ferns. We have seen earlier that there has been serious disagreements not only over the definition of the specific boundaries between *grisea* (Blanford) and *farinosa* (Forsk.), but also at the generic level, as to whether these species be referred to *Cheilanthes* Sw. or *Aleuritopteris* Fée. Copeland (1947) separates a group of 15 species from *Cheilanthes* Sw. and includes them in *Aleuritopteris* Fée on the basis of the shape of frond, ceraceous covering of the lamina and number of sporangia per sorus. These are all characters of the sporophyte. The naked gametophyte with a lateral meristem and asymmetrical development of the prothallus in the early stages becomes cordate shaped and prostrate in adult condition in *A. grisea*. These characters are shared more or less by the gametophytes of some of the other genera of the Gymnogrammoideae of Christensen (1938), (cf. Stokey, 1951 for reference).

Considering the role of the gametophyte in elucidating taxonomic problems, the present account of *A. grisea* is of significance. It is very much desired that study of the gametophyte of some related species of *Cheilanthes* Sw. be undertaken for purposes of comparison.

SUMMARY

A method for the culture of fern prothalli in an extensive scale is described. The gametophyte of *Aleuritopteris grisea* (Blanford) comb. nov. is naked without any setae, hairs, or glands and differentiates a lateral meristem which produces a somewhat asymmetrical prothallus at early stages, but the adult prothallus is cordate shaped and dorsiventral. The ontogeny of the sex organs has been outlined with more important details.

Grateful thanks are due to Mr. C. M. Bastia, M.Sc., Lecturer in Botany, Ravenshaw College, Cuttack, for his help in preparation of the Figures for publication.

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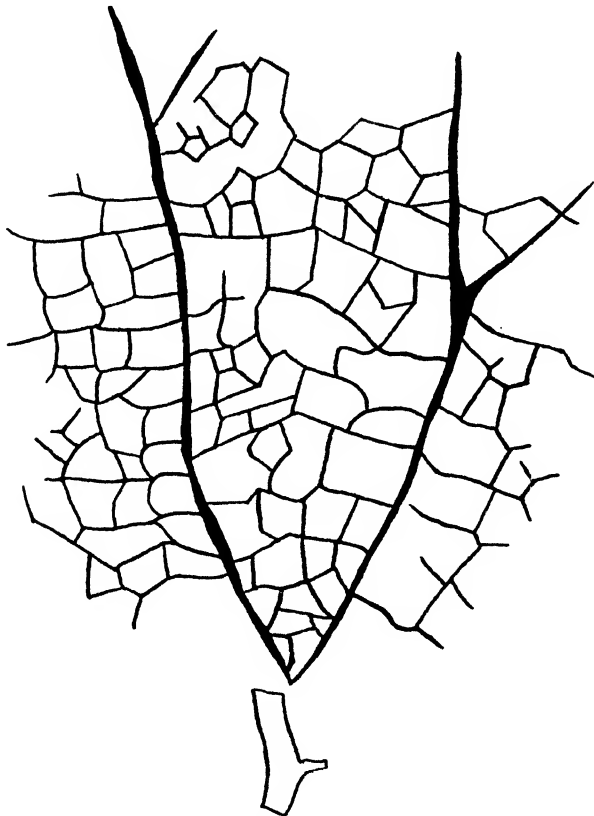
**HAUSMANNIA INDICA SP. NOV. GUPTA, A DIPTERIDACEOUS
LEAF FROM THE JURASSIC OF RAJMAHAL HILLS,
BIHAR (INDIA)**

by K. M. GUPTA, *Jaswant College, Jodhpur*

(Communicated by P. Maheswari, F.N.I.)

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The family Dipteridaceae is well represented in the Indo-Malayan flora and the genus *Dipteris* is commonly found in North India (Seward, 1914). The family, however, has not been recorded in India in the fossil condition, except for an undoubted specimen of *Hausmannia* discovered by the author several years ago at the now well known locality Nipania in the Rajmahal Hills, Bihar (Gupta, 1936). The present note is based on that material.



TEXT-FIG. 1. Camera lucida drawing of part of the leaf of *Hausmannia indica* sp. nov. Gupta showing reticulate venation, characteristic of the Dipteridaceae. \times ca. $9\frac{1}{2}$.

Hausmannia is a characteristic fern of the Jurassic and extends downwards into the Triassic and upwards into the lower Cretaceous (Halle, 1921; Seward, 1941). This typical fossil fern is believed by common consent to belong to the family Dipteridaceae (Seward, 1914) and known to occur all over the world including the Far East and south, namely Japan and Australia. It is also comparable to the living genera *Neocheiropteris* and *Cheiropleuria* but agrees more closely with *Dipteris*. The size of the leaves in different species of *Dipteris* varies greatly in area but they are essentially similar in their plan of construction; so also is the position in the few species that have been assigned to the fossil genus *Hausmannia*.

The new species from the Indian Jurassic, *Hausmannia indica* is represented by a single specimen in two counterparts (K/192 and K/193 Sahni Coll., Lucknow Univ.). The leaf is fragmentary and measures ca. 25×15 mm. (Pl. XV, Fig. 1). The venation is clearly preserved (Pl. XV, Fig. 2, text-fig. 1). There are two pairs of bifurcating veins which seem to converge at a point below in relation to an axis; but this convergence is not complete in the specimen. From these veins arise a number of secondary veinlets at almost right angles; these latter again give rise to tertiary branches which are still finer and join to form small rectangular areas, approximately 1×2 mm. in size.

The present specimen is undoubtedly referable to the genus *Hausmannia* in the wider sense in which it is used by Richter (1906) including both the Wealden species on which Dunker (1846) founded the genus and the Jurassic species on which Andrae (1885) founded the genus *Protoripis* (Andrae, 1885; Richter, 1906). Our specimen is sterile and too fragmentary to give a clear idea of the entire shape and size of the leaf and hence it is not possible to compare it with the Indian species of *Dipteris* (Beddome, 1892) or with the abnormal leaves of *Pleopeltis simplex* (Kashyap and Mehra, 1934). However, it can be compared with species of *Hausmannia* like *H. crenata* and *H. integrifolia* (Richter, 1906) as well as *H. ussuriensis* (Kryshtofovich, 1923; Kryshtofovich and Prynada, 1932). But it is rather difficult to compare with the eastern species described by Ôishi from the Rhaetic of Nariwa, Japan, such as *H. nariwaensis* and *H. dentata* (Ôishi, 1932) and *H. wilkinsi* Walkom from Cape York (Walkom, 1928). It has, however, some resemblances in the venation of leaves described by Carpentier from China (Carpentier, 1934), although it differs in size and presumably in its entire shape. The Rajmahal specimen is very fragmentary. However, in view of its rarity in the Indian rocks it seems advisable to give it provisionally a new specific name.

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Top  K/192 Salmi Coll. *Hausmannia indica* sp. nov. Gupta from the Jurassic of India. Impression of the leaf on the rock characteristic of the Rajmahal Series showing bifurcating veins and reticulum of veinlets. Almost natural size.

Fig. Same enlarged $\times 3$.

EMBRYOLOGICAL STUDIES IN COMPOSITAE

II. HELENIEAE

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(Communicated by P. Maheshwari, F.N.I.)

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INTRODUCTION

The present paper deals with the embryology of *Tagetes patula*, *Flaveria australasica* and *Gaillardia picta* which belong to the embryologically little investigated tribe Helenieae. Our previous knowledge of the tribe is limited to a few observations on *Tagetes signatus* by Dahlgren (1920) and on *Gaillardia pulchella* and *Gaillardia aristata* by Rosen (1944), who reported Fritillaria type of embryo sac development. In no other member of the tribe Helenieae embryo sac development has been described and Rosen's account of *Gaillardia* is rather meagre and does not include any observations on microsporogenesis, male gametophyte, development of the endosperm and embryo.

The materials of *Tagetes patula* and *Gaillardia picta* were collected from plants cultivated in the Botanical Garden of the Andhra University and that of *Flaveria australasica* was collected at Hyderabad by Mr. N. Ramaiah, College of Science, Osmania University. Formalin-acetic-alcohol or acetic-alcohol was used as fixative. Customary methods of dehydration and infiltration were followed. The sections were stained either with Delafield's haematoxylin or safranin and fast-green.

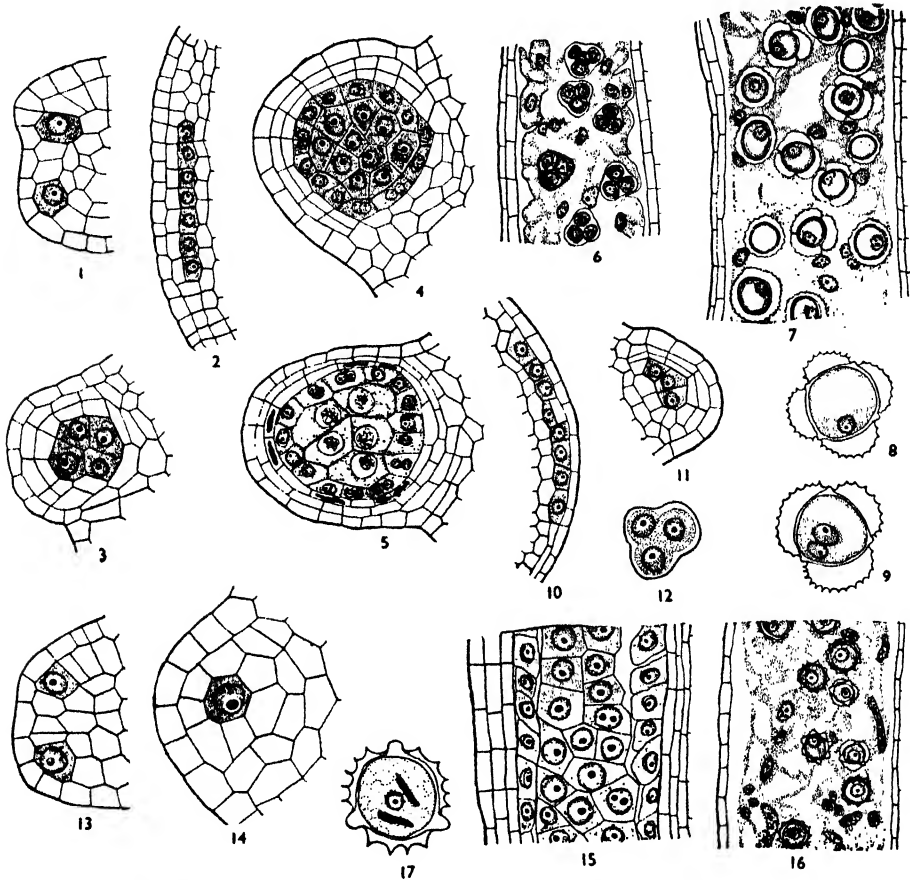
OBSERVATIONS

Organogeny.—Both bisexual and female flowers are found in the same inflorescence. In bisexual flowers the organogeny takes the following sequence: petals, stamens and sepals followed by gynoecium and in the female flowers the sequence is petals, sepals and gynoecium.

Microsporogenesis and Male Gametophyte.—The development of the anther and pollen is closely similar in all the three species investigated and resembles that described in *Launaea pinnatifida* (Venkateswarlu and Maheswari Devi, 1955). The structure of the anther shows the epidermis, two wall layers and tapetum surrounding the sporogenous tissue (Figs. 1-7, 10, 11, 13-16). Here also the fibrous endothecium is not differentiated.

A true periplasmodial type of anther tapetum is found (Figs. 6, 7, 16). Unlike *Launaea pinnatifida* where the nuclei of the periplasmodium degenerate soon, in the three Helenieae studied here they remain healthy for a long time (Figs. 7, 16). The primary sporogenous cells undergo a few mitotic divisions in all directions and give rise to a moderately extensive sporogenous tissue (Figs. 3-5, 11, 15). The pollen mother cells divide in a simultaneous manner. The two meiotic divisions take a normal course and bilateral as well as tetrahedral pollen tetrads are produced. Cytokinesis is by furrowing (Fig. 12).

The mature pollen grains are spherical, echinate and the spines have broad bases (Figs. 9, 17). Usually there are three germ pores, but in *Tagetes patula* a few

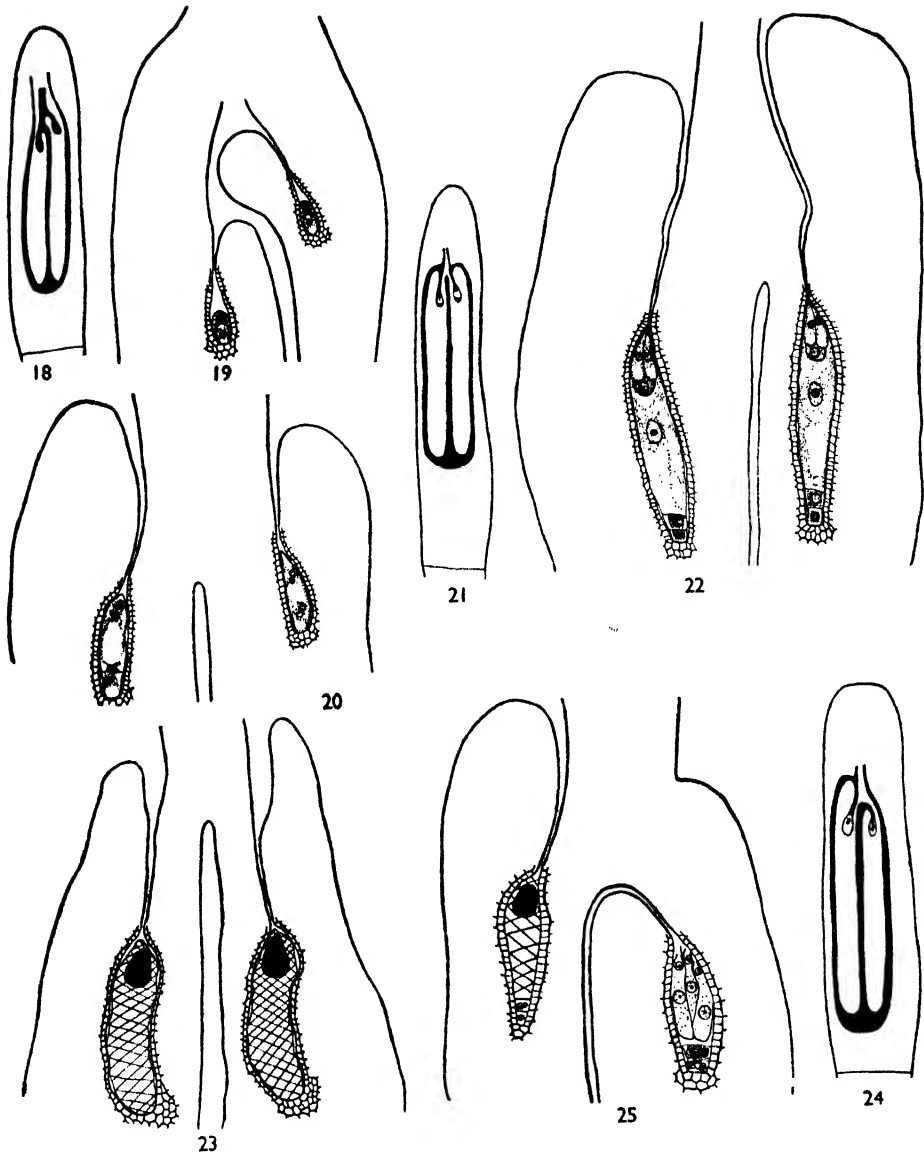


FIGS. 1-9—*Tagetes patula*. Fig. 1. T.s. of an anther half showing primary archesporium. $\times 707$. Fig. 2. L.s. anther lobe showing primary parietal layer and the primary sporogenous layer. $\times 527$. Figs. 3-5. Anther lobes at various stages of its development. $\times 707$. Figs. 6, 7. L.s. part of an anther lobe showing the periplasmodial tapetum surrounding the pollen tetrads and 1-nucleate pollen grains respectively. $\times 340$. Fig. 8. A 1-nucleate pollen grain showing four germ pores. $\times 707$. Fig. 9. A 2-nucleate pollen grain. $\times 707$.

FIGS. 10-12—*Flaveria australasica*. Fig. 10. L.s. anther lobe showing primary archesporium. $\times 388$. Fig. 11. T.s. anther lobe showing sporogenous tissue and two wall layers. $\times 527$. Fig. 12. Tetranucleate pollen mother cell showing cytokinesis by furrowing. $\times 707$.

FIGS. 13-17—*Gaillardia picta*. Fig. 13. T.s. anther half showing primary archesporium. $\times 527$. Fig. 14. T.s. anther lobe showing primary parietal and sporogenous layers. $\times 707$. Fig. 15. L.s. part of an anther lobe at the stage when the tapetal cells become binucleate. $\times 527$. Fig. 16. L.s. part of an anther lobe showing periplasmodial tapetum surrounding 1-nucleate pollen grains. $\times 340$. Fig. 17. 3-nucleate pollen grain. $\times 707$.

pollen grains with four germ pores such as reported in *Tridax procumbens* and *Blumea laciniata* by Banerji (1940, 1942) were encountered (Fig. 8). However,



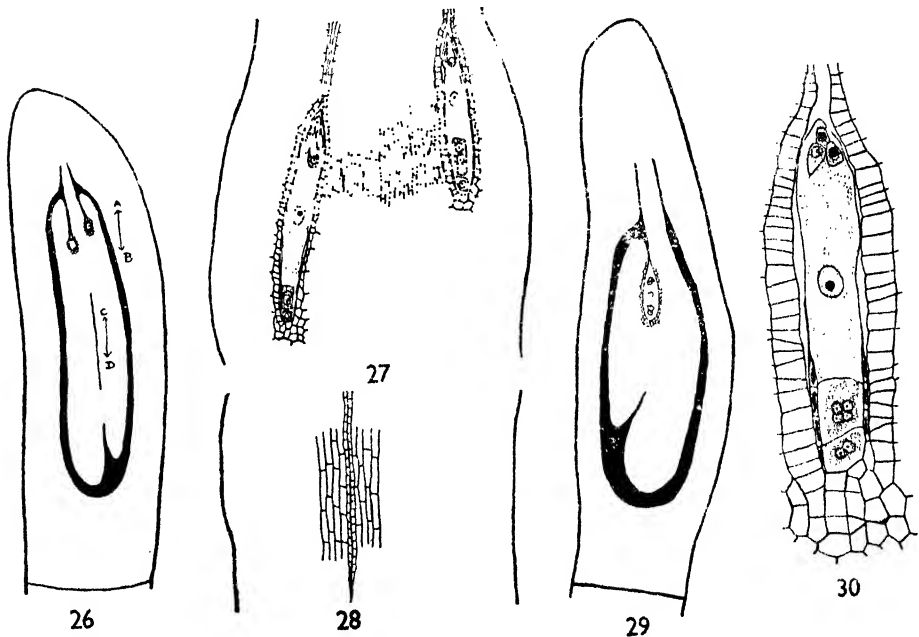
FIGS. 18-25—*Tagetes patula*. L.s. ovaries showing two ovules. Fig. 18. L.s. ovary showing two ovules facing each other. $\times 7$. Fig. 19. Part of ovules from Fig. 18 enlarged, a magaspore tetrad is developed in each of them. $\times 63$. Fig. 20. L.s. part of two ovules contained in the same ovary and showing a 4-nucleate embryo sac in each of them. $\times 107$. Fig. 21. L.s. ovary containing two ovules arranged back to back. $\times 5$. Fig. 22. The two ovules in Fig. 21 enlarged. These show a mature embryo sac in each of them. $\times 107$. Fig. 23. L.s. two ovules contained in the same ovary, both ovules containing cellular endosperm and advanced embryos. $\times 63$. Fig. 24. L.s. ovary with two ovules. The micropylar side of one ovule faces the funicle side of the other. $\times 10$. Fig. 25. The two ovules in Fig. 24 enlarged. One of these shows three embryo sacs in it, while in the other a young embryo and cellular endosperm are already formed. $\times 63$.

none with six germ pores such as reported in *Dahlia* by Wodehouse (1931) has been met with during the present study. The pollen grains are 3-nucleate at the time of shedding (Fig. 17).

Ovary and Ovule.—As in all other Compositae the ovary of *Tagetes patula*, *Flaveria australasica* and *Gaillardia picta* is bicarpellary, syncarpous, unilocular and inferior with a single basal ovule which is anatropous, unitegmic and tenuinucellate.

The integument is massive and about 5–8 celled thick at the sides and 11–16 celled thick in the basal region of the ovule in *Tagetes patula*, 4 or 5 celled thick at the sides and 8–10 celled in the basal region of the ovule in *Flaveria australasica* and 4–7 celled thick towards the sides and 6–9 celled in the basal region of the ovule in *Gaillardia picta* at the time when the archesporial cell is fully differentiated (Figs. 40, 57). In the advanced ovules the thickness of the integument both on the sides as well as in its lower region increases very considerably. In the fully developed seed the integument becomes completely crushed and becomes reduced to a thin membrane.

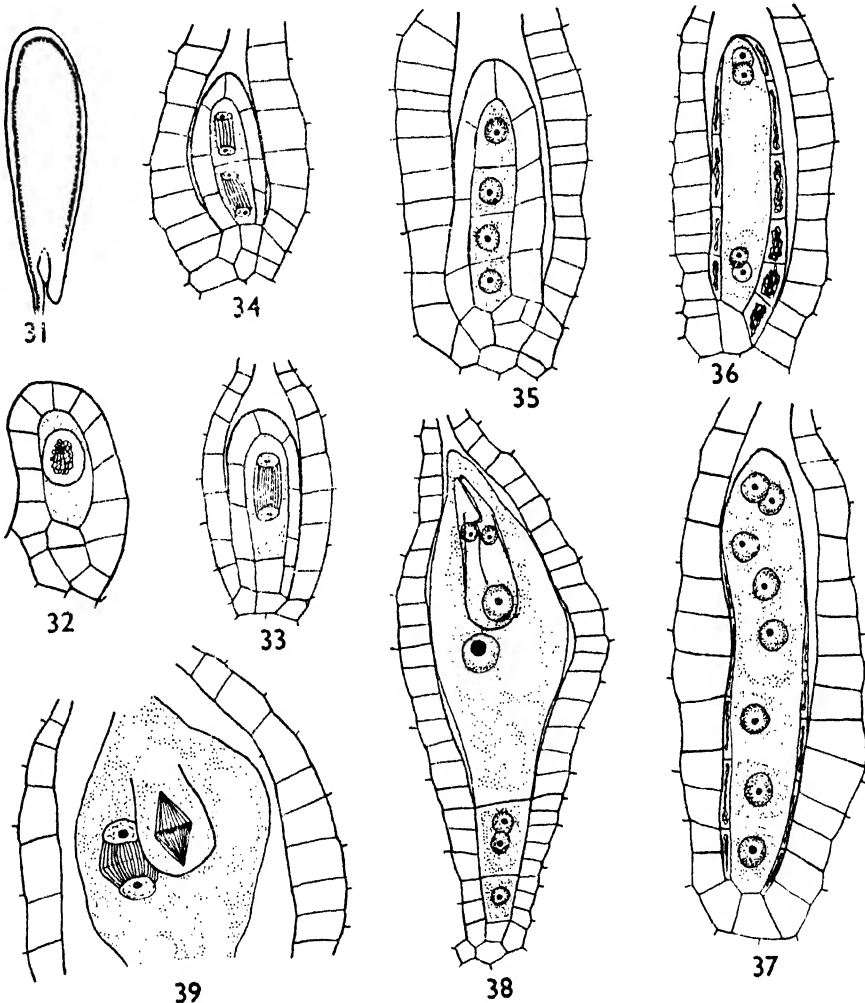
A feature of special interest is the exceptional occurrence of two ovules per ovary encountered in 14 out of 485 ovaries of *Tagetes patula* studied during the course of this investigation. In three of these the two ovules were seen to be partially fused while in the remaining eleven two separate ovules with the funicles fused at the base were found (Figs. 18–25). The two ovules in these cases were



FIGS. 26–30—*Tagetes patula*, cases of two ovules contained in the same ovary and showing fusion.

Fig. 26. L.s. ovary with two ovules fused back to back except at the base and a part in the middle. The two ovules, though fused, show one embryo sac in each. $\times 20$. Fig. 27. Part marked A-B in Fig. 26 enlarged to show details. $\times 100$. Fig. 28. Part marked C-D in Fig. 26 enlarged to show details. $\times 100$. Fig. 29. L.s. of ovary containing two ovules fused more intimately than in the case shown in Fig. 26, only one embryo sac is found in the fused structure. $\times 30$. Fig. 30. Embryo sac contained in the fused ovule shown in Fig. 29 enlarged to show details of structure. The egg apparatus is degenerating. $\times 320$.

found situated either back to back (i.e. funicles being adjacent to one other, Figs. 20-23) or face to face (the sides opposite to the funicles being adjacent to one other, Figs. 18, 19) or the funicle side of one ovule is adjacent to the side away from the funicle of the second ovule (Figs. 24, 25). In all these cases the embryo sacs and the embryos were found to develop normally (Figs. 18-23). Usually the stage of development of the embryo sac is the same in both the ovules (Figs. 18-23). However, in one case, one of the two ovules contained in the same ovary showed an embryo with octants, while, in the other, three fully formed embryo sacs were found (Figs. 24, 25).



FIGS. 31-39. *Flaveria australasica*. Fig. 31. Ovule showing integumentary vascular trace. $\times 15$. Figs. 32, 33. L.s. nucellus showing megaspore mother cell in meiotic prophase I and telophase I. $\times 790$. Fig. 34. Dyad cells in meiotic telophase II. $\times 790$. Fig. 35. Megaspore tetrad. $\times 790$. Fig. 36. 4-nucleate embryo sac with degenerating nucellar epidermis. $\times 790$. Figs. 37, 38. Young and mature embryo sacs respectively. Fig. 37 $\times 790$ and Fig. 38 $\times 436$. Fig. 39. The integumentary tapetum and the upper part of the embryo sac showing the first division of the fertilized egg and primary endosperm nucleus. $\times 790$.

In one case the two ovules in the same ovary were found in a completely fused state at the apex and the base and also for a considerable length in the middle. In the latter portion, on close examination, it was found that the outermost layer of the cells of the ovules were found to be in very close approximation with each other. The two ovules contained fully developed embryo sacs (Figs. 26-28).

Figs. 29 and 30 show a very interesting case. In the ovary sketched, there is a single structure which represents two ovules as can be seen from the two protrusions in its basal region and only one embryo sac is found in this structure. In this embryo sac the antipodals and the secondary nucleus are in a healthy condition, while the cells of the egg apparatus at the micropylar pole of the embryo sac are in a degenerating stage (Figs. 29, 30).

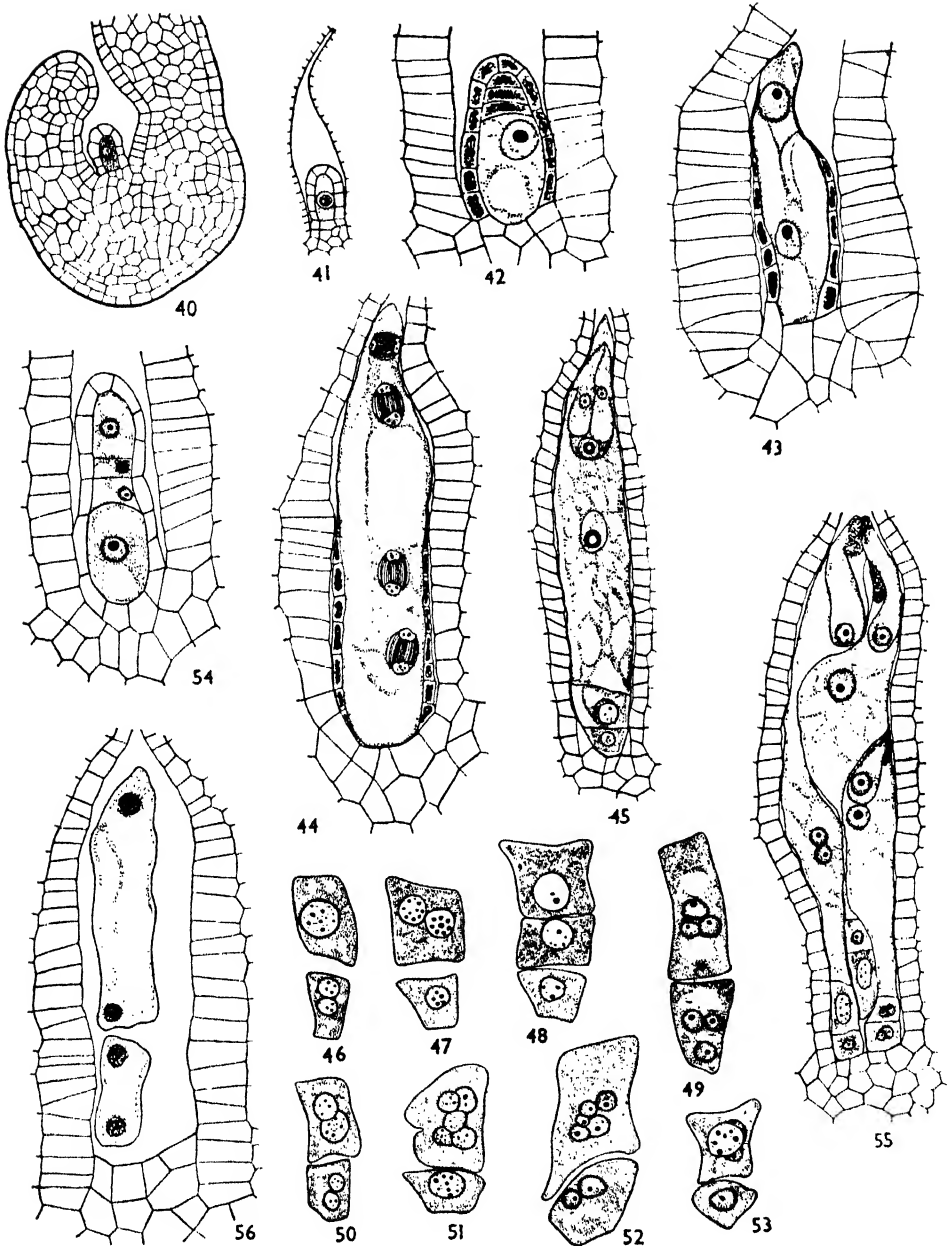
The ovules in the above exceptional cases also show integumentary vascular traces such as recorded in the ovules of the uniovulate ovary of *Tagetes patula* (Venkateswarlu, 1941). In *Gaillardia picta*, however, the ovule does not show an integumentary vascular trace.

Megasporogenesis and Female Gametophyte.—In *Tagetes patula* and *Flaveria australasica* the primary archesporium consists of a single hypodermal cell. It enlarges and becomes the megaspore mother cell without cutting off a parietal cell. A linear tetrad is formed after two meiotic divisions and the chalazal megaspore develops into the 8-nucleate embryo sac (Figs. 31-38, 40-45). The synergids are hooked (Figs. 38, 45). There is a great variation in the number of the antipodal cells and in the number of nuclei in an antipodal cell, a feature met with in many other members of Compositae such as *Eclipta erecta* (Bhargava, 1935), *Bidens leucanthus* (Dahlgren, 1920), *Zinnia grandiflorum* (Palm, 1931), *Xanthium spinosum* (Dahlgren, 1920), *Helianthus annuus* (Tackholm, 1916; Dahlgren, 1924), *Blumea laciniata* (Banerji, 1942), *Antennaria* and *Gnaphalium* (Schnarf, 1931), *Mikania scandens*, *Ageratum conyzoides* (Mitra, 1947), *Eupatorium cannabinum*, *Ageratum mexicanum*, *Erigeron*, *Solidago* and *Aster* (Schnarf, 1931). The number of the antipodal cells in *Tagetes patula* and *Flaveria australasica* varies from two to three. Where three antipodals occur, each of them is uninucleate (Figs. 37, 48). In such cases of *Flaveria australasica* when only two antipodal cells are present, one of them is binucleate. In *Tagetes patula*, when two antipodal cells are present, the number of nuclei in each cell varies from one to six (Figs. 46-53). Usually the antipodals persist even up to the formation of a mature embryo (Figs. 73, 74).

Usually only one embryo sac is developed in each ovule of *Tagetes patula*, but in a few cases two or three embryo sacs were found. No cases of ovules with more than one archesporial cell or megaspore mother cell were met with but sometimes megaspore tetrads with more than one megaspore developing further were encountered (Fig. 54). Therefore it is probable that the extra embryo sacs are formed due to further development of more than one megaspore of a tetrad. These double and triple embryo sacs show normal structure characteristic of the species and are found to be in a perfectly healthy condition (Figs. 54, 55). In one case with two binucleate embryo sacs in an ovule both the embryo sacs were found in a degenerating condition (Fig. 56).

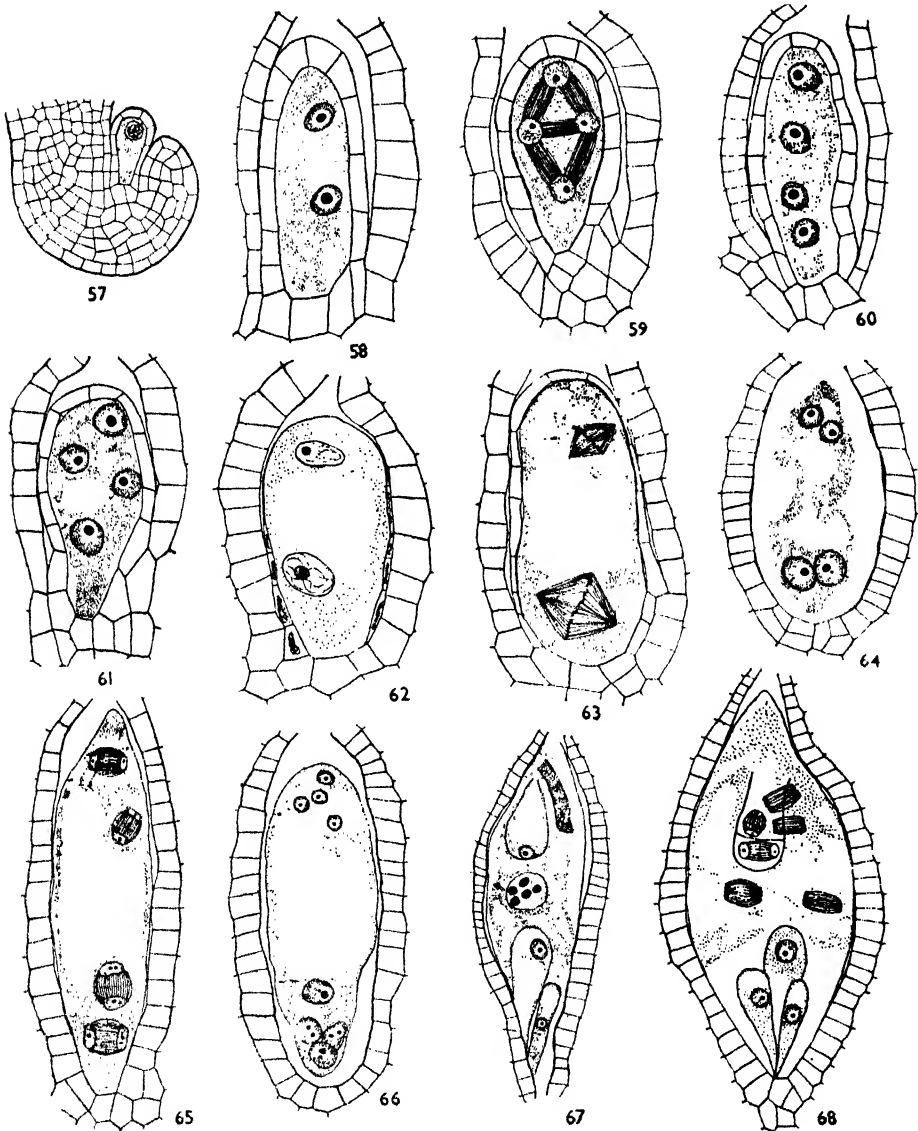
In *Gaillardia picta* the embryo sac develops according to the *Fritillaria* type. The megaspore mother cell undergoes the usual meiotic divisions unaccompanied by any wall formation and becomes tetranucleate. In some cases the four nuclei are arranged one at either pole and one on each side while in other cases they form a linear row (Figs. 57-61). Whatever may be the initial position they ultimately take 1+3 arrangement, three at the chalazal end and one at the micropylar end. These two groups are separated by vacuoles. All the four nuclei take part in the formation of the embryo sac.

The three nuclei at the chalazal end fuse together thus resulting in a secondary 2-nucleate embryo sac with a single large-sized triploid chalazal nucleus and a smaller haploid micropylar nucleus. The two nuclei then divide to give rise to the



FIGS. 40-56—*Tagetes patula*. Figs. 40, 41. L.s. young ovules showing primary archesporial cell and megaspore mother cell respectively. $\times 213$. Fig. 42. L.s. ovule showing a linear tetrad. The nucellar epidermal cells begin to degenerate. $\times 527$. Figs. 43-45. Stages in embryo sac development. Figs. 43, 44. $\times 527$ and Fig. 45. $\times 213$. Figs. 46-53. Variations in the antipodals. $\times 527$. Fig. 54. A linear tetrad with chalazal and micropylar megaspores developing further. $\times 527$. Fig. 55. Two 2-nucleate embryo sacs in the same ovule, both are degenerating. $\times 527$. Fig. 56. Three fully formed embryo sacs in an ovule. $\times 213$.

secondary 4-nucleate and then the 8-nucleate stage. Here we have four haploid nuclei at the micropylar end and four triploid nuclei at the chalazal end (Figs. 62-67). The nuclei of the micropylar quartet are smaller in size than those of the chalazal. Three of them form the egg apparatus while the fourth becomes the upper polar nucleus. The egg shows the characteristic basal nucleus and a terminal vacuole. The two synergids are pear-shaped and show a basal vacuole with the nucleus situated above it. From the lower quartet one of the nuclei migrates to the



Figs. 57-68—*Gaillardia picta*. Figs. 57-67. Various stages in the development of the Fritillaria type of embryo sac. Fig. 68. L.S. embryo sac showing young embryo, dividing endosperm nuclei and persistent antipodals. Fig. 57. $\times 194$; Figs. 58-63. $\times 527$; Figs. 64-67. $\times 336$ and Fig. 68. $\times 172$.

centre and forms the lower polar nucleus which later fuses with the upper polar nucleus resulting in a tetraploid secondary nucleus. The remaining three nuclei at the chalazal end form the antipodals. The central antipodal cell is egg-like in appearance while the other two resemble the synergids in their form. Thus the antipodals simulate the egg apparatus in appearance (Figs. 67, 68). Two of the antipodals persist till a few endosperm nuclei are formed, while the third, usually the centrally placed one, persists till even an advanced embryo is formed. Owing to insufficient material, Rosen described the structure and behaviour of the antipodals in *Gaillardia pulchella* and *Gaillardia aristata* in early stages only and most probably in these two species also they persist as in *Gaillardia picta*.

Fertilization.—In *Tagetes patula*, *Flaveria australasica* and *Gaillardia picta* fertilization is porogamous. The pollen tube passes between the egg and one of the synergids. The contents of the pollen tube are discharged into one of the synergids through a terminal pore. Details of the process are followed only in *Tagetes patula*. In the embryo sac the male gametes lose their rod-shaped form. They show a somewhat coiled thread-like structure surrounding a nucleolus-like body when they are within the egg cell and within the secondary nucleus (Fig. 69). The same has been recorded previously in *Crepis capillaris* by Gerassimova (1933). The nucleolus-like structure increases in size and gradually becomes applied to the egg nucleus (Fig. 70). Gradually the male and female nuclei fuse with each other and become indistinguishable as separate entities. Triple fusion and syngamy take place more or less simultaneously. In one case, however, triple fusion was completed earlier than syngamy (Fig. 70). After fertilization the embryo sac increases in size, while the synergids become more and more vacuolated.

In *Gaillardia picta* the secondary nucleus is tetraploid and after fusion with the male gamete it becomes pentaploid (Fig. 67). The pollen tube in *Tagetes patula* and *Gaillardia picta* persists till the first division of the fertilized egg is completed, while in *Flaveria australasica* it is not persistent and disappears.

Endosperm.—The first division of the primary endosperm nucleus is completed slightly earlier than that in the fertilized egg and is not accompanied by the formation of a cell wall.

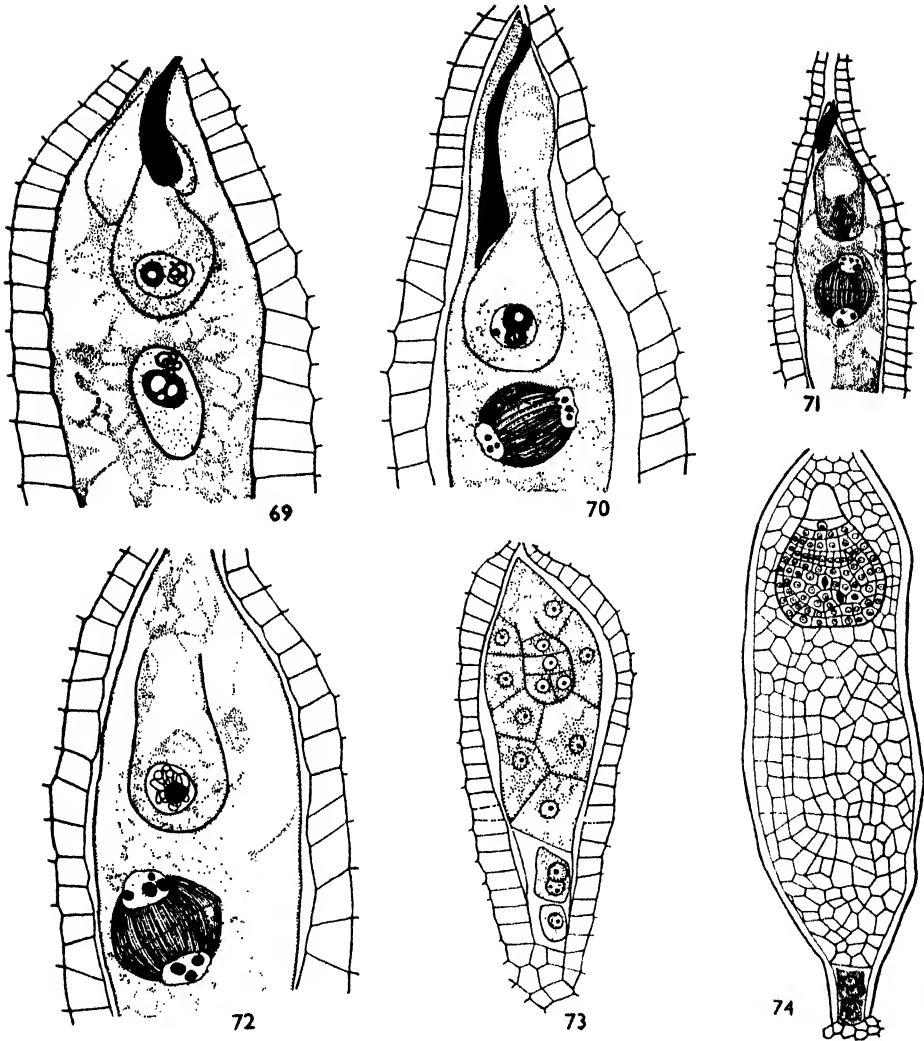
In *Tagetes patula* the orientation of the spindle of the first division of the primary endosperm nucleus is not constant. In some cases it is transverse and in others it is oblique, sometimes being very nearly vertical (Figs. 70–72). In *Flaveria australasica* the long axis of the spindle is parallel with that of the embryo sac and no variation in its orientation has been found (Fig. 39).

The endosperm becomes cellular in later stages (Figs. 73, 74) and is completely consumed by the embryo in the fully developed seeds in all three species studied.

Embryo.—The embryo development in *Tagetes patula*, *Flaveria australasica* and *Gaillardia picta* is essentially similar except for some differences in detail.

The first division of the fertilized egg is transverse (Figs. 39, 75, 86, 87, 95) and a two celled proembryo is formed. The terminal cell of the two celled proembryo, *ca*, divides vertically resulting in two juxtaposed cells while the basal cell, *cb*, divides transversely forming two cells which are arranged in a superposed manner. The upper of these is termed as *m* and the lower, i.e. the one towards the micropylar side, as *ci* (Figs. 76, 96, 97). In *Gaillardia picta*, *ca* divides slightly earlier than *cb* (Figs. 86, 87).

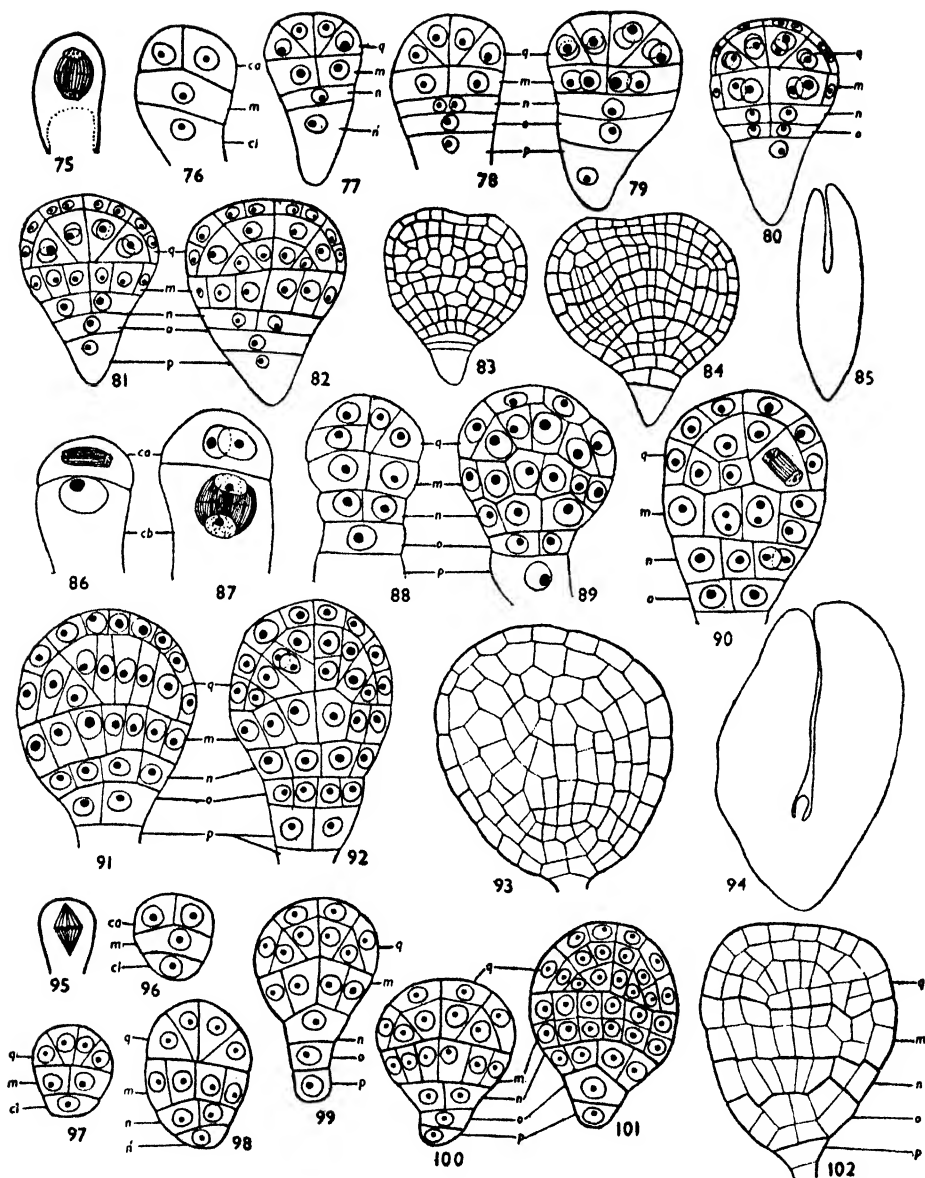
The two cells derived from *ca* undergo a vertical division each, the walls being laid at right angles to that formed in the preceding division. The four circum-axially arranged cells derived from *ca* form the tier *q*. The next division in this tier results in the formation of octants and walls are obliquely oriented (Figs. 77, 88, 98). In *Gaillardia picta*, however, occasionally the walls in one of the quadrants may be transverse (Fig. 88). Periclinal divisions take place in the octants resulting in the demarkation of a single layer of dermatogen cells (Figs. 80, 89, 99). The cells inner to the dermatogen divide further in all directions and the derivatives



FIGS. 69-74—*Tagetes patula*. Fig. 69. Syngamy and triple fusion. $\times 527$. Fig. 70. A section of upper part of embryo sac at the time of syngamy and at the time of the first division of the primary endosperm nucleus. $\times 527$. Figs. 71, 72. L.s. part of the embryo sacs at about the time of the first division of the fertilized egg and the endosperm primordium. $\times 231$ and $\times 527$ respectively. Fig. 73. L.s. embryo sac showing young embryo and cellular endosperm and persistent antipodals. $\times 231$. Fig. 74. Same as Fig. 73 but at an advanced stage. $\times 150$.

later on give rise to periblem and plerome. The dermatogen cells, however, divide further only in an anticlinal manner. The derivatives of the tier *q* give rise to the two cotyledons and the stem tip (Figs. 82-85, 92-94, 99-102).

The cell *m* divides into two juxtaposed cells by the time octants are formed in the tier *q* (Figs. 77, 88, 97). By another vertical division at right angles to the first one, quadrants are formed in this tier also. Close upon the differentiation of dermatogen in the tier *q*, periclinal divisions result in the formation of dermatogen in the tier *m* also (Figs. 80-82, 89-92, 98-100). In *Gaillardia picta*, however,

Figs. 75-85—*Tagetes patula*.Figs. 86-94—*Gaillardia picta*.Figs. 95-102—*Flaveria australasica*.

Various stages in the development of the embryo. Figs. 75-82. $\times 527$; Figs. 83-84. $\times 213$; Fig. 85. $\times 47$; Figs. 86-92. $\times 527$; Fig. 93. $\times 258$; Fig. 94. $\times 50$; Figs. 95-101. $\times 527$ and Fig. 102. $\times 388$.

dermatogen in tier *m* is differentiated slightly later (Fig. 89). Periclinal divisions take place in the cells inner to dermatogen in the tier *m* ultimately resulting in the differentiation of periblem and plerome in it (Figs. 82-85, 89-94, 100-102). These periclinal divisions are followed by anticlinal divisions. The derivatives of the

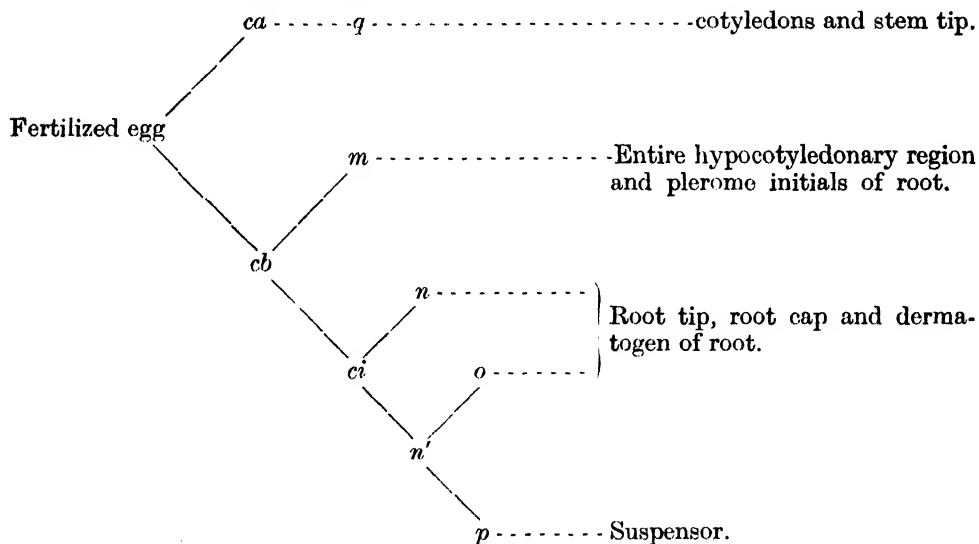
tier *m* give rise not only to the entire hypocotyledonary region but also to the plerome initials of the root.

The cell *ci* divides transversely and gives rise to two superposed cells *n* and *n'* (Figs. 77, 98). Of these the former undergoes two vertical divisions at right angles to each other and the derivatives contribute to the formation of the root cortex and lateral portions of the root cap (Figs. 80-84, 89-93, 100-102). The cell *n'* divides by a transverse wall and forms two superposed cells, namely *o* and *p* (Figs. 78-83, 88-92, 99-102). The cell *o* (forming the tier *o*) at first undergoes two vertical divisions at right angles to each other (Figs. 80, 89-92). The derivatives from the cells of this tier contribute to the formation of the root cap and completion of the dermatogen of the root.

The cell *p* undergoes one or two transverse divisions resulting in a short suspensor. These cells sometimes divide vertically (Figs. 84, 90-93). The suspensor is short in all the species studied.

From the foregoing it can be made out that both *ca* and *cb* contribute to the development of the embryo proper and that the embryo development in *Tagetes patula*, *Flaveria australasica* and *Gaillardia picta* conforms to the Asterad type. The cell *m* (superior daughter cell of *cb*) gives rise to the entire hypocotyledonary region and also to plerome initials of the root and therefore the embryogeny in the three species keys out to the Senecio variation of the Asterad type (Johansen, 1950).

The relation of the individual cells of the proembryo to the organs of the mature embryo is shown in the following schematic representation:



Seed.—In fully developed seeds of *Tagetes patula*, *Flaveria australasica* and *Gaillardia picta* the endosperm and almost all layers of the integuments are consumed and the embryo lies within a thin membrane closely appressed to the pericarp. The innermost layer of cells of the pericarp in *Gaillardia picta* as also the cells of the outer layer of the integument contain prismatic crystals, but as pointed out already the latter is completely crushed in mature seeds.

SUMMARY

Three members of the tribe Helenieae, namely *Tagetes patula*, *Flaveria australasica* and *Gaillardia picta*; have been studied.

The sequence of development of the floral whorls is corolla, androecium, calyx and gynoecium.

The anther shows an epidermis, two wall layers, amoeboid anther tapetum of parietal origin and moderately extensive sporogenous tissue. No fibrous endothecium is differentiated. The pollen mother cells divide in a simultaneous manner and cytokinesis is by furrowing. The pollen grains are shed in the 3-nucleate stage. The exine is echinate and shows three germ pores. A few cases of pollen grains with four germ pores have been encountered in *Tagetes patula*.

Usually there is a single, unitegmic, tenuinucellate, anatropous ovule in an ovary but about 3 per cent of the ovaries of *Tagetes patula* examined showed two ovules which sometimes showed various degrees of fusion. An integumentary vascular trace occurs in the ovules of *Tagetes patula* and *Flaveria australasica*.

In *Tagetes patula* and *Flaveria australasica* the female archesporium is 1-celled. A linear tetrad is formed and the chalazal megaspore develops into an 8-nucleate embryo sac. The number of antipodals varies from two to three and the number of nuclei in an antipodal varies from one to six in *Tagetes patula* and one to two in *Flaveria australasica*. Double and triple embryo sacs occur in *Tagetes patula*, probably owing to the further development of more than one megaspore of a tetrad.

In *Gaillardia picta* the development of the embryo sac is of the Fritillaria type. There is a secondary 2-nucleate stage followed by a secondary 4-nucleate stage. The antipodals are persistent.

Fertilization is porogamous. Syngamy and triple fusion take place more or less simultaneously. Pollen tubes are persistent in *Tagetes patula* and *Gaillardia picta*.

Endosperm is of the nuclear type. It later becomes cellular and is completely consumed in the mature seed.

Embryo development conforms to the Asterad type and keys out to the Senecio variation.

In fully developed seeds the endosperm and the integument are completely consumed except for a thin membrane.

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TENDRILS OF THE CUCURBITACEAE: THEIR MORPHOLOGICAL NATURE ON ANATOMICAL EVIDENCES

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INTRODUCTION

All but one genus, *Ecballium*, of the family Cucurbitaceae are characterized by the possession of a simple or variously branched extra-axillary tendril. Its morphological nature has attracted the attention of workers from the early part of the nineteenth century. Different authors working on different materials have tried to interpret the morphology of this organ but they never agreed to a common explanation, e.g., Müller (1887) interpreted it as 'axis plus leaf', Green (1905) and Majumdar (1926) as 'branch', Trinkgeld (1923) as 'leaf', Green (1905) again as 'stipule', and so on. Lately Miss Khansaheb (1947) revived interest in this organ of climbing. She studied anatomically the tendril and the node bearing it in a number of cucurbits, and found that the tendril received its vascular supply both from the leaf-trace bundles (outer ring) and the cauline bundles (inner ring) of the axis. This led her to the conclusion that the tendril is a *leaf-stem complex*.

In order to find out the true morphological nature of this climbing organ anatomical studies of the nodes and vascular supplies to this organ (tendril) of twenty-four species of the Cucurbitaceae under fourteen genera were undertaken and the results and conclusions are recorded in this short report.

MATERIALS AND METHODS

The following twenty-four species were collected from Dacca town and its suburbs:

Species	Vernacular name	Habitat
1. <i>Benincasa hispida</i> Cogn.	Chalkumra	Cultivated
2. <i>Bryonopsis laciniosa</i> Naud.	Mala	Wild
3. <i>Citrullus colocynthis</i> (Linn.) Schrad.	Mâkâl	Wild
4. <i>Citrullus vulgaris</i> Schrad.	Tarmuj	Cultivated
5. <i>Coccinia indica</i> (Naud.) Wight and Arn.	Telâkuchâ	Wild
6. <i>Cucumis sativus</i> Linn.	Sashâ	Cultivated
7. <i>Cucumis melo</i> Linn.	Futi	Cultivated
8. <i>Cucumis</i> sp.	X	Wild
9. <i>Cucurbita maxima</i> Dachesne	Mithâkumrâ	Cultivated
10. <i>Gymnopetalum cochinchinensis</i> Kurz.	X	Wild
11. <i>Lagenaria vulgaris</i> Seringe	Lâu	Cultivated
12. <i>Luffa cylindrica</i> (Lour) Roem.	Dhoondol	Cultivated and Wild
13. <i>Luffa acutangula</i> Roxb.	Jhingâ	Cultivated
14. <i>Luffa graveolens</i> Roxb.	X	Wild

Species		Vernacular name	Habitat
15.	<i>Momordica charantia</i> Linn. ..	Uchchhe	Cultivated
16.	<i>Momordica cochinchinensis</i> Spreng. ..	Kâkrol	Cultivated
17.	<i>Momordica dioica</i> Roxb. ..	Bankâkrol	Wild
18.	<i>Momordica</i> sp. ..	X	Wild
19.	<i>Mukia maderaspatana</i> Kurz. ..	X	Wild
20.	<i>Thladiantha calcarata</i> Clarke ..	X	Wild
21.	<i>Tricosanthes anguina</i> Linn. ..	Chichingâ	Cultivated
22.	<i>Tricosanthes cucumerina</i> Linn. ..	Banchichingâ	Wild
23.	<i>Tricosanthes dioica</i> Roxb. ..	Patol	Cultivated
24.	<i>Zanonia indica</i> Linn. ..	X	Wild

The material was fixed in F.A.A. and washed thoroughly before use. Transverse sections were made, both free-hand and with hand-microtome. For the study of nodal anatomy the following technique was adopted: 1.5% hot agar solution was smeared evenly on a slide. The slide was then placed over a warm plate with water underneath it, so that the fixing material did not solidify before the sections were placed and arranged serially. After the operation the slides were removed from the warm plate and the fixing material was allowed to cool down to fix the sections in proper positions. When the glue dried the sections were stained either differentially with safranin and fast green and made permanent, or stained only in safranin, mounted in glycerine solution and sealed with a paraffin preparation.

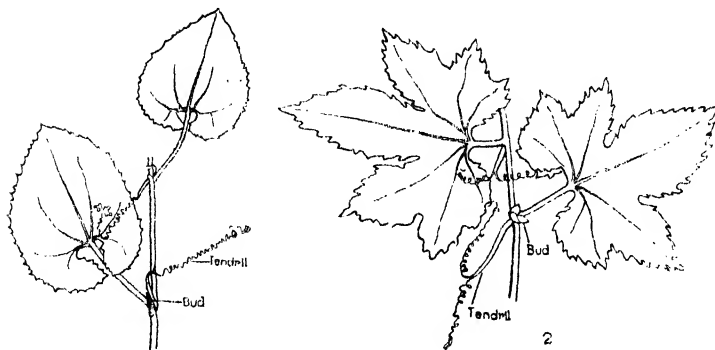
To study the vascular disposition at the node and its leaf, bud and tendril the former was cleared in the following way:—first of all the somewhat hard epidermis was peeled off with a pair of fine forceps avoiding any damage to the vasculature. Then the nodal piece was warmed with a small quantity of concentrated HCl for five to seven minutes, then 30 to 35 c.c. of 10% NaOH solution was poured slowly down the test tube and warmed for two to three minutes; all the soft tissues precipitated down leaving the vascular system intact with some soft tissues still attached. This was taken out of the test tube, rinsed thoroughly in water; stained in dilute safranin and again washed in water; the attached soft tissues were then removed with a mounted needle and a fine camel hair brush. The cleared and stained vascular system was then mounted in 40–45% glycerine and sealed. Figures were drawn with the help of a camera lucida.

ABBREVIATIONS USED

The following abbreviations have been used: *L*—leaf; *L'*—1st lateral leaf-trace bundle; *L''*—2nd lateral leaf-trace bundle; *M*—median leaf-trace bundle; *T*—Tendril; *T*¹—1st tendril-trace bundle; *T*²—2nd tendril-trace bundle; *B*—bud; *b*—bud-trace bundles; 1, 2, 3, 4, 5 refer to vascular bundles of the outer and 6, 7, 8, 9 and 10 refer to vascular bundles of the inner rings, of the axial stele. The same number has been retained for these bundles in the axial rings of different internodes; supply of the outer bundles from those of the inner is indicated by a number in brackets, the number refers to the bundle of the inner ring.

OBSERVATIONS

The tendrils in the Cucurbitaceae may be simple (Fig. 1) or branched (Fig. 2). Each of them is differentiated into a comparatively strong basal portion which remains erect and an upper part which is coiled.



TEXT-FIG. 1

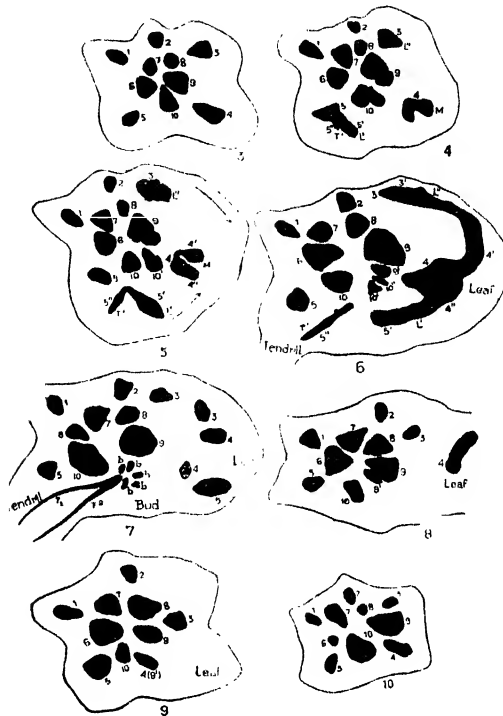
FIG. 1. Habit sketch of *Thladiantha calcarata* Clarke. ($\times \frac{1}{2}$); FIG. 2. Habit sketch of *Bryonopsis laciniosa* Naud. ($\times \frac{1}{2}$).

Anatomy of the node.—The vascular bundles of the node, like those of the internode, are unequal and dissimilar. In the internode the ten bundles are arranged in two rings; those of the outer ring (Fig. 3—1, 2, 3, 4, 5) are smaller in size and extent than those of the inner one (Fig. 3—6, 7, 8, 9, 10). But in some species the vascular bundles of the two rings in the node come so close to one another that it becomes rather difficult to assign them to their respective rings. In the present description of the nodal anatomy the bundles will, however, be referred to as if they are arranged in two rings with these assigned numbers.

A. *Vascular supply of the leaf.**—Each leaf receives three bundles (3, 4, 5) all of which come from the outer ring at the node. The bundles of the outer ring are, therefore, regarded as leaf-trace bundles. The median bundle remains simple in *Bryonopsis* (Figs. 11, 12), *Coccinia*, *Lagenaria*, *Benincasa*, but it becomes deeply cleft in the form of 'U' on its way to the base of the petiole of the leaf in *Thladiantha* (Figs. 4, 5) and in *Citrullus*, *Cucumis*, *Cucurbita*, *Gymnopetalum*, *Luffa*, *Momordica*, *Mukia*, *Tricosanthes*, and *Zanonia*. The median bundle (4), simple or lobed, goes as a whole to supply the leaf leaving a gap in the outer stellar ring. The gap, thus formed, is then occupied by a bundle branched off from one of the larger inner bundles, 9 in the case of *Thladiantha* and 10 in the case of *Bryonopsis*, which also supplies traces to the bud (Figs. 9, 15).

The first lateral bundle (L') of a leaf is formed by the branching of bundle 5 of the outer ring flanking the median bundle (Figs. 4–6 and 11–12). The second lateral (L'') may be formed as in the case of the first lateral, from a part of bundle 3 on the other side of the median, e.g. in *Benincasa*, *Coccinia*, *Luffa*, *Momordica*, *Thladiantha* (Figs. 5–6), or the whole bundle 3 may go to supply the leaf as its second lateral, as in *Bryonopsis* (Figs. 12–15), *Citrullus*, *Cucurbita*, *Cucumis*, *Gymnopetalum*, *Lagenaria*, *Mukia*, *Tricosanthes* and *Zanonia*. When the entire bundle goes to supply the leaf as a lateral its position in the next internode is taken by a branch of bundle 9 of the inner ring, which also takes part in the vascular supply to the bud (Figs. 13–15). In this respect the second lateral and its reconstitution at the next higher internode takes place exactly in the manner of the median trace bundle.

* The course of the bundles of the outer and inner rings have been followed through two contiguous internodes and the intervening node. Figures in serial transverse sections have been given for only two representative species, *Thladiantha calcarata* Clarke, and *Bryonopsis laciniosa* Naud. In all the figures the median and the two lateral bundles are marked 4, and 3, 5 (outer ring) respectively.



TEXT-FIG. II

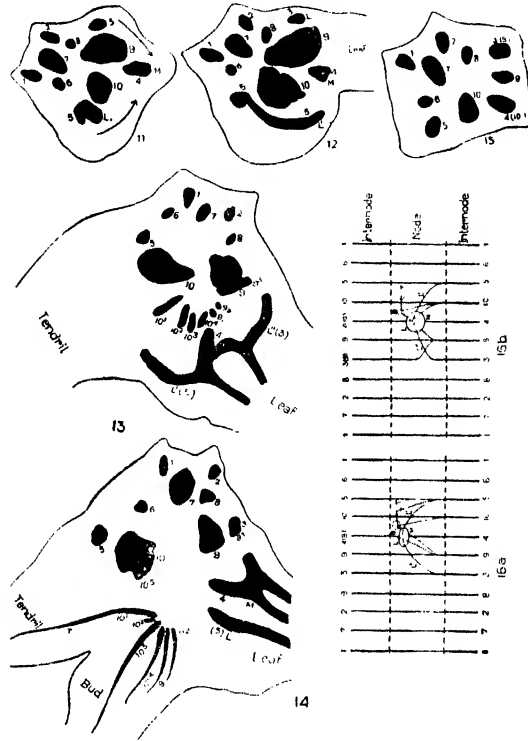
FIGS. 3-9. Serial transverse sections of one internode to the base of the next higher internode through a node of *Thladiantha calcarata* Clarke showing the vascular supplies to leaf bud and tendril. ($\times 12$). For explanation of Fig. 10, see Text-fig. III.

The origin and vascular supply to a Cucurbita leaf may be summarized as follows:—

- (i) The stelar system (axial) consists generally of ten vascular bundles arranged alternatively in two rings.
- (ii) Three bundles from the outer ring contribute to the formation of the three-bundled trace of each leaf.
- (iii) One entire bundle constitutes the median and goes to the leaf causing a gap in the outer ring of axial bundles, which is later filled up at the next higher internode by a branch coming from a much larger bundle of the inner ring. This larger bundle also sends a branch to the axillary bud (Figs. 4-9 and 13-15).
- (iv) The first lateral is always a branch of the vascular bundle of the outer ring placed immediately lateral to the median (Figs. 4-6 and 11-13).
- (v) The second lateral may be either a branch of the other proximal bundle of the outer ring as in the case of the first lateral as in *Thladiantha* (Figs. 5-6), or the whole bundle may go to supply the leaf, when a branch from a bundle of the inner ring comes to occupy its place in the outer ring as in *Bryonopsis* (Figs. 11-15). This bundle of the inner ring also sends out a branch to the bud (cf. the case of the median).

B. *Vascular supply of the bud*.—Normally the bud traces branch off from bundles 9 and 10 of the inner ring (Figs. 6, 7 and 13, 14) which also send out branches

to occupy the gaps in the outer ring caused by the departure of the lateral and median traces of the axillant leaf. These bundles of the inner ring enlarge before they give out the bud traces (Figs. 6-7 and 12-14). The vascular supplies to the bud thus come exclusively from the bundles of the inner ring (described cauline) as their branches.



TEXT-FIG. III

FIGS. 10-15. Serial transverse sections of one internode to the base of the next higher internode through a node of *Bryonopsis laciniosa* Naud. showing vascular supplies to leaf, bud and tendril. ($\times 12$). FIGS. 16a and 16b. Diagrammatic representations of the course of vascular bundles from one internode to the next internode through a node of *Thladiantha calcarata* Clarke and *Bryonopsis laciniosa* Naud. respectively.

C. *Vascular supply of the tendril.*—According to the nature and mode of vascular supply from axial ring of bundles the tendrils may be grouped under two general types, namely (a) the *Bryonopsis* type, and (b) the *Thladiantha* type. In the first type the tendril supply is derived exclusively from the bud trace which in turn is formed by the branches coming from bundles 9 and 10 of the inner ring. Thus here the tendril receives all its vascular supply directly from the stelar system of the axillary bud (Figs. 13, 14). This category includes the tendrils of *Bryonopsis*, *Benincasa*, *Citrullus*, *Coccinia*, *Cucumis*, *Cucurbita*, *Lagenaria*, *Momordica*, *Mukia*, *Tricosanthes* and *Zanonia*.

In the '*Thladiantha*' type, on the other hand, each tendril receives two bundles one of which comes from the bud trace (9', 9'', 10', 10'') while the other from one of the laterals (5') of the leaf trace (Figs. 5-7). Thus the second type of tendril receives its vascular supply both from the bud stele and a lateral trace of the axillant leaf.

DISCUSSION AND CONCLUSION

The tendril of the Cucurbitaceae has attracted the attention of various workers from the nineteenth century, and quite a number of divergent views on its morphological nature have so far been put forward.

Müller (1887) examined anatomically a number of tendrils and came to the conclusion that in both simple and compound tendrils the non-sensitive base is a shoot axis and the upper portion a leaf-spindle, i.e. the tendrils are *axis plus leaf*. But in *Cucumis* he found the tendril to be entirely *foliar* in nature.

Engler (1904) who collected specimens with thorn-like appendages, one of which grew out into a tendril, regarded the tendril as a *stipule*.

Green (1905) made the following suggestions to explain the nature of the tendrils in the Cucurbitaceae: the tendril is (i) one of a pair of stipules, the other being suppressed; (ii) extra-axillary branch at the side of a leaf; (iii) if the extra-axillary bud develops into a flower, then the tendril is peduncular in origin, i.e. one of the flower stalks becomes modified and adnate to the leaf-stalk and appears as a tendril.

According to Green, Naudin holds that a branch arises in the axil of a leaf; and that in the case of a simple tendril it becomes completely merged with the petiole, or with stem and grows no further, the only sign of this branch is one leaf which it bears on one side but which is reduced to the mid-rib: *This is the tendril*. Compound tendril, according to him, is formed by the elongation of the branch which bears several leaves that are modified into tendril branches. Green also quotes Payer according to whom the tendril is formed by the splitting of the petiole.

Braun and Wydler (quoted in Rendle, 1952) regarded a simple tendril as one of the bractlets of the axillary flower, while in a compound tendril each branch was believed to be a rib of a simple leaf.

Goebel (1905), who does not accept anatomy as evidence, regards simple tendril as prophyll of axillary shoots of which only one developed, whereas branched tendrils are axes which bear leaves transformed into branches of the tendrils.

Hägerup (1930) regarded all compound tendrils to be modified shoots, and in *Cucumis* it is the first prophyll of the secondary axis.

Sawhney (1919-20) on the basis of vascular contribution regarded arms of branched tendrils and upper part of the simple tendrils as homologous with leaves, and the basal part with shoot axis.

Trinkgeld (1923) supports Müller in all his interpretations, namely, simple tendril of *Cucumis* is entirely foliar; in simple tendril of other cucurbits basal part is a transformed shoot axis, upper portion terminal leaf, and in branched tendrils basal portion is the shoot axis, and branches are metamorphosed leaves. This interpretation is based on the analogy of the arrangement of vascular bundles which is in a ring in the axis, and dorsiventral in the leaf, and also on the distribution of sclerenchyma which is in a ring in the axis, but as bundle-caps in the leaves.

Majumdar (1926) from specimens of *Cucurbita maxima* Dachesne collected by him supported the shoot nature of the branched tendril, i.e. axis plus leaves modified.

Khansaheb's (1947) interpretation is based on a study of the anatomy of nodes, and the vascular supply to the tendril. She found that tendrils in her specimens received vascular supplies from bundles of both the inner and outer rings of the axial stele (cf. *Thladiantha* type, Figs. 4-6), and on the basis that the vascular bundles of the outer ring are leaf-trace bundles and those of the inner ring are cauline bundles (cf. Sawhney, 1919-20) she interpreted tendril as a *leaf-stem complex*.

The results of the present studies are recorded in the foregoing pages; and according to the nature of the vascular supply from the axis the tendrils have been divided into two major types, namely, (1) the *Bryonopsis* type, and (2) the *Thladiantha* type.

In the tendrils of the first type, represented by those of *Bryonopsis laciniosa* Naud., the vascular supply comes entirely and directly from the bud trace. From

the nature of the origin of vascular supply, the tendrils in these cases cannot be identified with leaf. The presence of sclerenchyma in the form of a closed ring in the cucurbits is a stem feature, therefore it cannot be the petiole or the ribs of a leaf. It cannot be regarded as a bud also, as its origin is not connected with a leaf, and its vascular supply is also different from the normal mode of supply to a bud in these species as well as in the majority of dicotyledons.

The tendril of the first category is, therefore, regarded as an *outgrowth of the bud axis*. Such outgrowths are not unusual, and Arshad Ali (1955) and Fattah (1955) have shown respectively that the tendrils of the Vitaceae and the inflorescence axes of the Solanaceae are outgrowths of the axes which bear them.

In the second category the tendrils receive vascular supply partly from the vascular system of the bud axis and partly from a branch of the lateral trace bundle destined to supply the axillant leaf. On the dictum of Sinnott and Bailey (1914), any organ at the base of the leaf getting vascular supply from the branch of a lateral leaf-trace bundle should be regarded as a stipule. This has been supported by later workers (Mitra and Majumdar, 1952). Therefore, the tendril of this category should be interpreted as *stipule-stem complex*.

NATURE OF THE VASCULAR BUNDLES IN THE STELAR RINGS

The axial stele consists of ten vascular bundles arranged in two rings, five in the outer and five in the inner. The bundles of the outer ring have been described as 'leaf-trace bundles' and those of the inner ring as 'cauline bundles'. My studies of the vascular system of the axis done in serial transverse sections through two consecutive internodes and the included node show that the vascular supplies to the leaf, bud and the tendril at each node are derived directly and indirectly from the bundles of the inner ring.

When a bundle of the outer ring goes to supply a leaf, a branch from one of the inner ring bundles comes to take its place in the next higher internode. But the bud receives all its vascular supply from the bundles of the inner ring. Therefore, the bundles of the inner ring not only supply the bud traces but also send out branches to supply the leaf. It is to be noted that the bundles of the inner ring never bodily shift to the outer ring. The bundles of the outer ring are, therefore, really leaf-trace bundles passing through an internode on their way to the leaves higher up in the axis. The bundles of the inner ring may be called 'cauline bundles' in the sense that they never leave the axis as a whole causing gaps in the axial cylinder; they only send out branches to the leaf and axillary bud. It will be interesting to note how these bundles end in the apical meristem of an adult shoot.

The dual nature of the internodal bundles, i.e. foliar (leaf trace) and cauline (axial), indicates the dual nature of the axis, namely, the outer mantle served by the leaf-trace bundles, and the core served by the cauline bundles. If this interpretation is correct then it supports the *mantle-core* theory of Hofmeister (1851), Saunders (1922) and revived by Mitra and Majumdar in 1952.

SUMMARY

The morphological nature of the tendrils of the Cucurbitaceae has been studied anatomically. According to the nature of vascular supply they are described as:—(1) an 'Outgrowth of the bud axis' when its vascular supply comes only from the bud trace, and (2) 'Stipule-stem complex' when its vascular supply is derived from branches of both the bud trace and of a lateral of the trace of the axillant leaf. 20 out of 24 species studied have their tendrils supplied by bud stele alone. The bundles of the outer ring are really leaf-trace bundles and those of the inner ring cauline.

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It is the greatest pleasure of the author to acknowledge his indebtedness to Prof. G. P. Majumdar, F.N.I., under whose guidance this piece of work was carried out.

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CONTRIBUTIONS TO OUR KNOWLEDGE OF INDIAN FRESHWATER PLANTS

PART 2. ON SOME ASPECTS OF THE HABIT, STRUCTURE, LIFE-HISTORY AND AUTECOLOGY OF *LIMNANTHEMUM CRISTATUM* GRISEB. AND *LIMNANTHEMUM INDICUM* THW.*

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INTRODUCTION

Limnanthemum cristatum Griseb. and *L. indicum* Thw. (family Gentianaceae) are widely distributed throughout India, but very little is known regarding their general morphology and autecology, apart from brief general descriptions in various systematic works (cf. Roxburgh, 1824; Bentham, 1869; Hooker, 1890; Prain, 1903; Biswas and Calder, 1937). Srinivasan (1942) has given a detailed account of the internal morphology of the flower, while Mukherjee (1951) has dealt with its cytology. In the present paper detailed descriptions of the two species, their habit, their external morphology, reproduction as also internal morphology and some of the important physico-chemical conditions of *L. cristatum* are described.

HABIT

Gentianaceae, a large family of herbaceous plants, is interesting owing to the range of its geographical distribution, which is from the extra-tropical regions to the hottest part of the tropics, and its habitat. The plants of this family are mainly terrestrial but according to Arber (1920) such marsh plant as *Menyanthes* might form a transition to the typically aquatic genus *Limnanthemum*.

According to Roxburgh (1824), Hooker (1834), Bentham (1869), Prain (1903) and Rendle (1938) species of *Limnanthemum* (*L. cristatum*, *L. indicum*, *L. pellatum*) are all free floating aquatics. Hooker (1890) states that some species like *L. nymphaeoides*, *L. cristatum*, *L. indicum* root at the nodes; *L. aurantiacum*, *L. forbesianum* do not root at the nodes and *L. parvifolium* has stems apparently rooted on mud. According to Biswas and Calder (1937) *L. cristatum* is a rooted aquatic, *L. indicum* has long, floating stems, rooting at the nodes and *L. parvifolium* is apparently rooted on mud at the base. In the course of the present study the author observed that both *L. cristatum* and *L. indicum* are predominantly rooted aquatics, the roots being present on the main tuber as well as at the nodes (Figs. 1 and 2). Only for a short period in their life-history they live as floating aquatics. In these species, each runner that comes out from the main underground rhizome, bears at its apex a fresh rhizome. These floating rhizomes develop roots by which they later attach themselves to the soil, become underground, and give rise to fresh runners. Some-

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times, before the floating rhizomes could attach themselves to the soil, they get detached from the main body of the plant probably by wind action or some mechanical disturbance, float up and behave as floating aquatics. This process is repeated by successive generations of rhizomes and very often a number of such free floating plants could be observed in ponds.

To verify whether the plants when detached can live free floating for a considerable length of time, observations were carried out on healthy growing *L. cristatum* in a pond having a depth of 6 to 8 ft. at the centre. Some runners with fully developed young plants at their ends were severed from the mother plants and allowed to float freely within an artificial enclosure made in the deeper part of the pond so that they did not get the opportunity of coming into contact with the bottom mud. A number of young plants were also left in the pond * outside the enclosure as control. From the 5th to 6th day the leaves and roots of plants in the enclosure started decaying. From the 15th to 17th day they started sinking and between 18th and 20th day all of them sank to the bottom. These observations were repeated with confirmatory results. The young plants which were left in the tank as control, without being enclosed, reached the shallower regions and rooted themselves. The experiment was repeated with a few plants of *L. indicum* brought from Kaushalya Ganga, Puri and identical results were obtained. The plant is usually found as rooted aquatics in ponds with the depth of water varying from 3 to 8 ft., the length of the runners often ranging from 2½ to 7 ft. depending on the depth of the tank.

It may be concluded from the above observations that *L. cristatum* and *L. indicum* are both rooted aquatics and that they float only for a limited period of their life-histories. But, if by any chance, a free floating plant is unable to anchor itself to the soil within a limited time, it perishes.

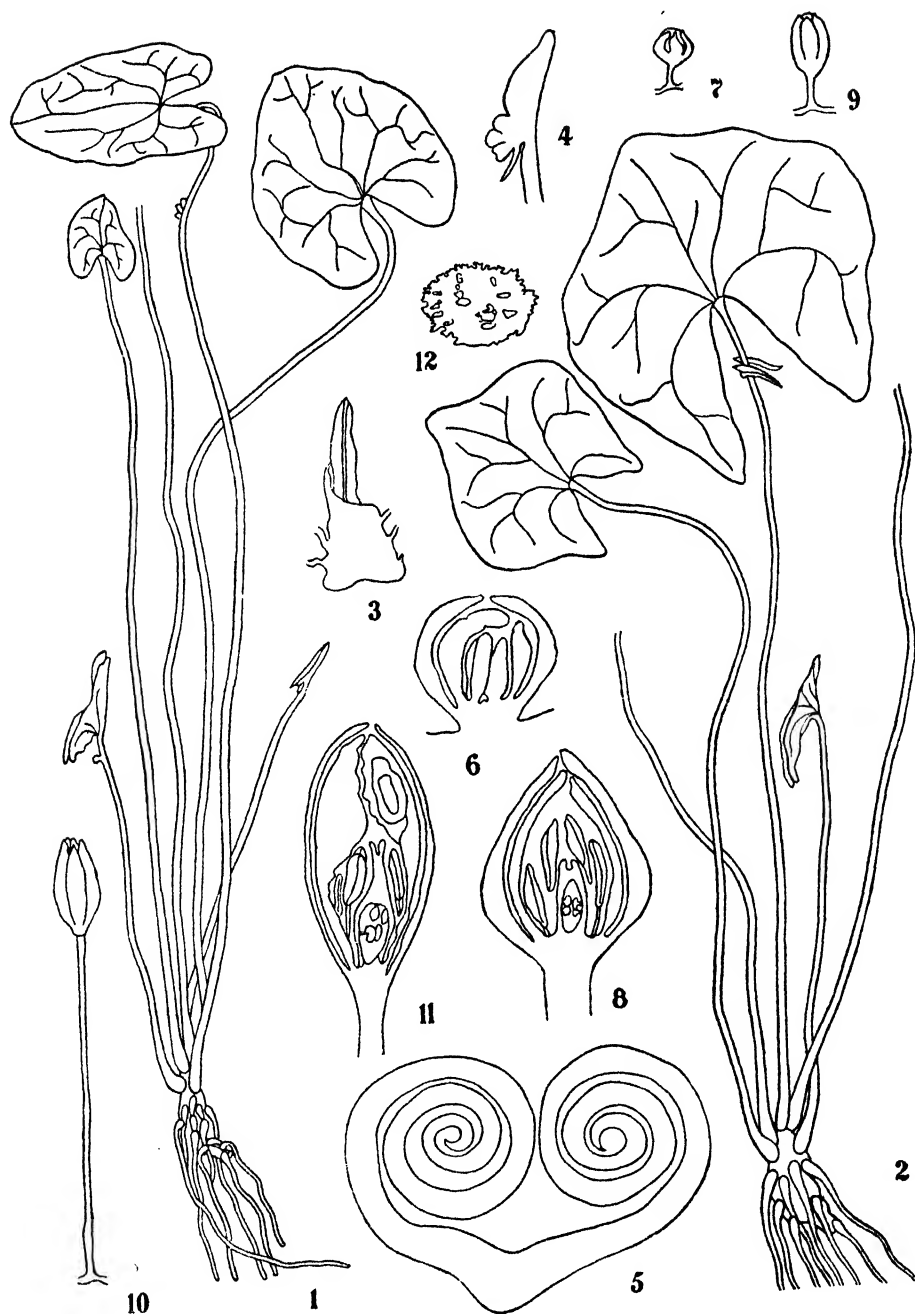
EXTERNAL MORPHOLOGY

The underground tuber has a thick, hard, protective bark-like covering which is absent when the tuber is exposed. The roots develop from the base of the tuber within the soil. The runners come out from that portion of the tuber which is jutting out of the soil. These runners, floating while attached to the rooted tuber on one side, are sometimes very long and develop foliage leaves at their tips. The inflorescence develops on the runner, a little below the foliage leaf giving it the appearance of a lateral outgrowth from the leaf, the entire runner appearing as the petiole. The shoot morphology was first described by Goebel (1891-93) and later by Arber (1920) who states 'In plants of *Limnanthemum*, examined at the flowering season, it is found that a long stalk given off from the rhizome appears to bear both a lamina and flowers or, in other words, that the flowers seem to arise laterally from a leaf stalk. In reality this long stalk is, however, the axis of the inflorescence. The petiole has a short, sheathing base, which in youth surrounds the inflorescence. In development, the foliage leaf pushes the growing point to one side and comes to occupy the terminal position. Goebel considers that this peculiar mode of growth confers a definite biological advantage. The breadth of the leaf surface resting on water gives the inflorescence the necessary support, while the elongated inflorescence axis forms a substitute for both the elongated petiole and peduncle.'

The runners developing from the floating tubers are very thin, delicate and light green in colour. The fully formed runners developing from underground tubers are generally short and thick, ranging from 10.7 to 21.4 mm. in length.

Each leaf is entire, orbicular or deeply cordate developing independently on the runners. In *L. cristatum* the leaves are thinner with prominent venation whereas in *L. indicum* they are thicker with indistinct veins. In both the species the

* The experiment was carried out in a nursery pond at Cuttack (Orissa).



FIGS. 1—*Limnanthemum cristatum* Griseb. Plant $\frac{1}{2}$ Nat. size; 2—*Limnanthemum indicum* Thw. Plant $\frac{1}{2}$ Nat. size; 3—Growing point of the leaf coming out of the sheathing base ($\times 25$); 4—Leaf primordium enclosed within a sheath-like structure ($\times 25$); 5—Involute vernation of young foliage leaf ($\times 57$); 6—L.S. of early rudiment of flower bud ($\times 57$); 7—Flower bud with the pedicel developed ($\times 57$); 8—L.S. of flower bud with stamens and carpels ($\times 214$); 9—A fully formed bud ($\times 57$); 10—A bud with fully formed pedicel ($\times 57$); 11—L.S. of a fully formed bud ($\times 214$); 12—A seed ($\times 214$).

leaf primordium is about 1 mm. long (Fig. 3) and first develops on the rhizomes within a colourless, spathe-like structure with the primordium of the flower buds just at the base of the small folded lamina (Fig. 4). Below the rudiment of the inflorescence the runner elongates and comes out of the spathe. With this the leaf also increases in size and gradually opens out. In the bud the vernation of the lamina is involute (Fig. 5). When the lamina opens out it reveals 5 veins. The length of the lamina of *L. cristatum* varies from 35 to 93 mm. and width from 61 to 135 mm.; whereas in *L. indicum* it varies from 90 to 125 mm. and 150 to 175 mm. respectively.

In both the species the mature roots developing from underground tuber are 130 to 170 mm. in length and 10 to 12 mm. in width. In *L. cristatum* the adventitious roots that are present at the base of the floating rhizome are 3.4 to 14.8 mm. in length and 1.0 to 4.0 mm. in width. None of the roots possess root caps.

REPRODUCTION

Both the species reproduce vegetatively as well as sexually. But vegetative reproduction is more frequent and abundant than sexual reproduction.

Vegetative reproduction continues almost throughout the year but more vigorously from February to the end of June. Development of vegetative buds on the underground, rooted tubers, which form runners and then develop at their ends, floating tubers. These tubers float only as long they are attached to the rooted parent plants by runners, later they break away from the runner by some external agency and float away. Also very commonly vegetative buds are formed on the floating tubers and in the axils of leaves. These buds individually develop into runners and thus help in the ramification of the plants on which they are formed. Each of these runners again bear in turn on the free ends small tubers with leaves and inflorescence. These floating tubers when come in contact with substratum send out roots and each continues to exist as an independent, rooted plant. Thus repeated branching by means of vegetative buds enable a plant to explore the surrounding expanse of water with a close mosaic of leaves on the surface of the water. According to d'Almeida (1928) *Limnanthemum* is a type of water plant which is intermediate in habit between the *Nymphaea* type which is rooted in the mud and the floating type whose roots do not penetrate the soil but hang freely in the water. Sexual reproduction is carried on by seeds.

DEVELOPMENT OF THE INFLORESCENCE AND FLOWERS

As mentioned elsewhere in this paper, in both the species the inflorescence develops on the runner just at the base of the petiole. The inflorescence in both these species is of the racemose type and may be described as a corymb with a very short main axis as shown by d'Almeida (1928). In *L. cristatum* the early rudiment of the flower bud, when about 1 mm. in length, is almost round in shape and without a stalk. Longitudinal sections of this rudiment show only the primordia of sepals, petals and stamens (Fig. 6). It is without a stalk even when 1.5 mm. in length. When 2 mm. in length a pedicel which is about 1 mm. long is also developed (Fig. 7). Longitudinal section of this pedicelled bud shows the fully developed stamens and the carpels which have just started development with rudimentary ovules (Fig. 8). The pedicel elongates further to about 2 mm. It does not elongate further till the bud attains a length of about 3 mm. (Fig. 9). The bud thereafter elongates gradually to about 5 mm., while the pedicel grows rapidly to about 18 to 19 mm. (Fig. 10). In the fully formed bud the characteristic shape of the petals and the stamens with clearly differentiated filaments and anthers are easily seen (Fig. 11). In the flowers the length of the pedicel varies from 16 to 45 mm. No observation is on record as to how many days it takes from the first stage of flowering up to the formation of

seed and germination of those seeds. In nature the flowers of aquatic plants after being pollinated shed their petals, then the pedicels bend and go in the deeper layers of water, where the seeds mature and get dispersed. In a pond due to the presence of innumerable plants and also many other factors the study on the formation and germination of seeds is hampered very much. So studies on this aspect were conducted under controlled conditions.

A flower normally takes 33 to 43 days from the initial stage up to seed formation. From the first stage of flower formation to the opening up of the floral parts takes 15 to 18 days. The flowers remain in full bloom for 3 to 4 days, at the end of which, only the petals fall off. After the shedding of the petals the ovaries gradually increase in size and after 5 to 6 days the pedicels bend down carrying the mature ovaries with the persistent sepals in the deeper layers of water. Then after 10 to 15 days the ovaries dehisce and the seeds are dispersed. Each ovary might be having 5 to 6 ovules but generally only one seed is fully formed. Sometimes two seeds are also found in an ovary but that is very seldom. When the petals of flowers are shed, cloth bags are tied round the mature ovaries and the seeds are collected in the cloth bags. After the seeds have collected in the cloth bags they are immediately planted in a small earthenware tub having 1 inch sterilized soil at the bottom and 2 inches of water. Each seed is globular in shape with a minute stalk (Fig. 12). On the body of the seed very small, spinous projections are present. The length of the stalk varies from 0.25 to 0.5 mm. Seeds are 1.5 to 2.0 mm. in diameter.

The flower of *L. cristatum* is pentamerous (Figs. 13 and 14). In *L. indicum* the number of floral parts varies from 5 to 7 (Figs. 15, 16 and 17).

External morphology of fully developed flowers of *L. cristatum* and *L. indicum* is as follows:—

	<i>L. cristatum</i>	<i>L. indicum</i>
<i>Calyx</i> :	Sepals 5, united just at the base, free above with rounded apex, green with reddish margins (Fig. 18). Aestivation in bud imbricate.	Sepals 5 to 7, united at the base, green, acute apex, aestivation imbricate (Figs. 16 and 17).
<i>Corolla</i> :	Petals 5 (Fig. 19) joined at the base forming a very short tube, free above, valvate in bud and alternating with the sepals. Colour white except at the base where it is light yellow, each petal 7 to 10 mm. long, 5 to 8 mm. broad. A longitudinal fold present down the middle of each petal. Nectaries present on the corolla in 3 rows (Fig. 20). Outer row consists of white, hairy filaments at the mouth of the tube of the corolla. Middle one is a cluster of light yellow coloured, glandular bodies, which extend from the base up to almost the mouth of the tube and alternating with the filamentous nectaries. The third row is at the base of the tube and consists of small, white filaments. The two layers of cells in the centre of each filament have thickened	Petals 5 to 7, gamopetalous with a short, yellow, tubular portion at the base and 5 to 7 free lobes alternating with the sepals, upper parts of free lobes hairy. Aestivation valvate in bud. Nectaries absent.

*L. cristatum**L. indicum*

walls surrounded by a layer of bigger cells, only the outer walls of which are thickened (Fig. 21).

Androecium: Stamens 5, alternating with the petals, epipetalous, about 2.0 and 2.5 mm. long (Fig. 22). Anthers bilocular with short, linear filaments.

Gynoecium: Carpels 2, united, superior, unilocular ovary (Fig. 23). In young flowers the stigma is sessile and directly on the beak of the ovary. Green in colour with its apical portion bifid. In mature flowers the beak of the ovary elongates and curves slightly, bringing the free lobes of the stigma on one side of the ovary (Figs. 24 and 25). Placentation marginal (Fig. 26). The ovules are anatropous (Fig. 27). Ovules 6 to 10 in number, colourless when young, brown when half mature (Fig. 28), black when fully mature as seeds, which are spherical and have very thick testa (Fig. 29).

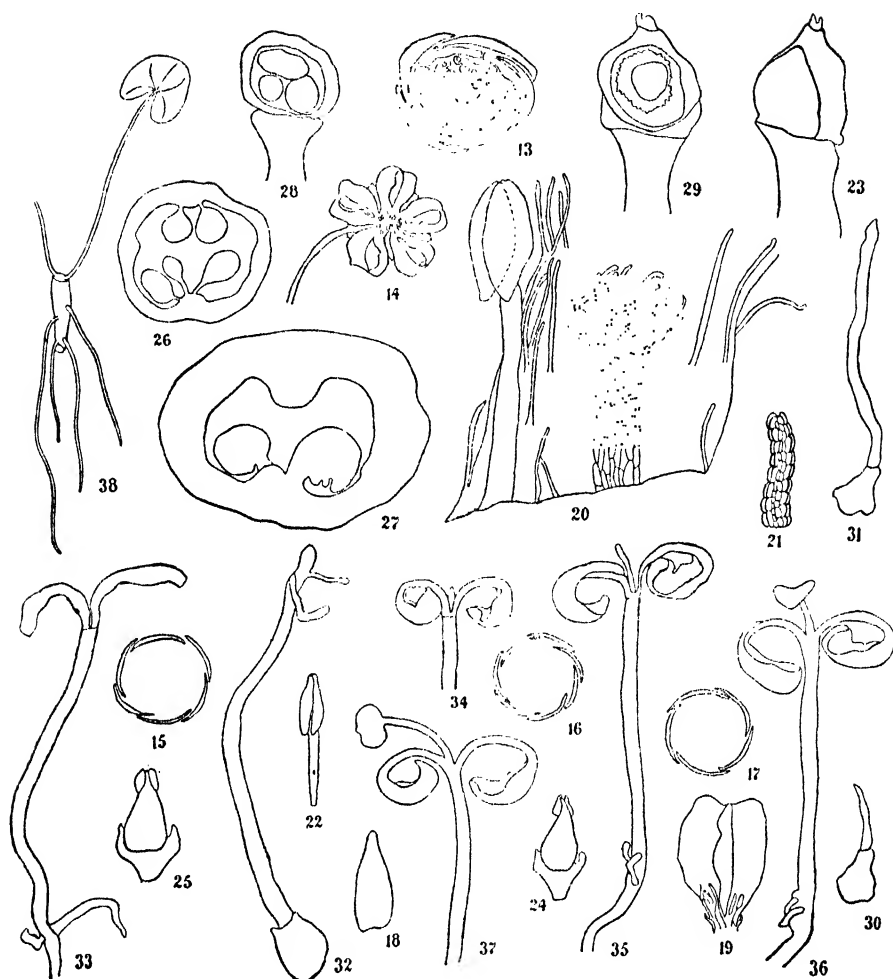
Stamens 5 to 7, epipetalous, united with the petals just at the base, alternating with the petals, anthers versatile and bilocular.

Carpels 3, united, superior, ridged, unilocular ovary. Stigma sessile, trifold. Placentation parietal. Ovules and seeds similar to those of *L. cristatum*.

GERMINATION OF SEEDS IN *L. CRISTATUM*

Twenty-five seeds were planted in a small earthenware tub, 6 inches in diameter, with 1 inch of sterilized soil and 2 inches of tap water. The earthenware tub was kept inside the laboratory, where the sunrays fell on it during the morning hours only, and the remainder of the day it was in the shade. The pond mud was sterilized by drying it stone hard in the strong sun for 2 weeks and then the normal temperature was brought back by keeping it in the laboratory within the small tub for a few days. During this period the mouth of the tub was covered with a cloth tied round it, so that the soil was not infected with any other external element. Later when the seeds were available they were planted in that tub.

Out of 25 only one seed germinated on the 92nd day. When germination started the seed was covered with mud and the hypocotyl was just protruding from the soil (Figs. 30 and 31). On the fifth day of the growth of the hypocotyl the small thin roots developed just below the hypocotyl apex (Fig. 32). Beyond the root the hypocotyl continued to elongate and on the 8th day morning the cotyledonary leaves opened out from the tip of the hypocotyl, hence it showed an epigeal germination. Up to this stage of development the seedling was in contact with the soil. Later the same afternoon the seedling broke off just below the roots from the seed and continued to survive as a floating aquatic (Fig. 33). On the 10th day morning a tip was found to develop in between the two cotyledonary leaves (Fig. 34). On the 12th day the tip had elongated more in size (Fig. 35). On the 15th day the foliage leaf though small in size was fully formed (Fig. 36). From the 17th day the seedling behaved as a rooted aquatic instead of a floating one (Fig. 37). So when the seed germinated the seedling behaved as a rooted aquatic for 7 days,



FIGS. 13—Floral diagram of *L. cristatum* ($\times 214$); 14—Flower of *L. cristatum* ($\times 57$); 15, 16, 17—Floral diagram of *L. indicum* ($\times 214$); 18—Sepal of *L. cristatum* ($\times 57$); 19—Petal of *L. cristatum* ($\times 57$); 20—Epipetalous stamen and 3 rows of nectaries ($\times 214$); 21—Filament of a nectary ($\times 214$); 22—A stamen ($\times 57$); 23—An ovary with the persistent sepals removed ($\times 214$); 24, 25—Mature ovaries ($\times 57$); 26—Placentation ($\times 59$); 27—Anatropous ovules ($\times 312$); 28—L.S. of half mature ovary ($\times 214$); 29—L.S. of mature ovary with one seed ($\times 214$); 30—Hypocotyl coming out of seed ($\times 12$); 31—Hypocotyl elongated ($\times 12$); 32—Roots developed a little below the hypocotyl apex ($\times 12$); 33—Hypocotyl elongated and the apex unfolding the cotyledons ($\times 12$); 34—Foliage developing in between the cotyledons ($\times 12$); 35—Foliage leaf more developed ($\times 12$); 36—Foliage leaf unfolded ($\times 12$); 37—Foliage leaf fully formed ($\times 12$); 38—*L. cristatum* grown on sand. Nat. size.

from the 8th day it behaved as a floating aquatic for 9 days and on the 10th day when root, foliage all were present the seedling started to behave again as a rooted aquatic.

Bertha Chandler (1910) worked on the germination of the seeds of *Utricularia emarginata* Benj. and had shown that the seeds flourished best in a shallow pan, having a thin layer of mud at the bottom with enough water to cover the mud. She had also shown that the best method of cultivation was to keep the plant in partial shade in still water.

Out of 25 seeds that were planted in the earthenware tub only one showed germination under similar conditions as shown by Chandler (1910), and which underwent no long dormant period. But the rest of the seeds had not shown germination and might have been in a dormant condition. Observations are being continued on their behaviour. Parija (1934) had shown that the seeds of Water Hyacinth remained dormant for at least one season, that is, November to June, and retained their viability for several years. Stiles (1936) writes that A. Fischer observed that mature seeds of a number of water plants such as *Sagittaria sagittifolia*, *Alisma plantago-aquatica*, *Hippuris vulgaris* and various species of *Potamogeton*, *Scirpus* and *Sparganium* did not germinate in pure water but did so readily in impure water containing bacteria.

INTERNAL MORPHOLOGY OF *L. CRISTATUM*

The vegetative parts were fixed in formal acetic alcohol and after passing through the usual xylol-alcohol grades were embedded in paraffin and 10μ thick transverse and longitudinal microtome sections were cut. For the anatomical study the sections were stained in safranin and light green. Camera lucida drawings have been made.

I. Root

1. T.S. of mature floating root—Fig. 39.

Epidermis—One layered, thin walled cells.

Hypodermis—One layered and parenchymatous.

Cortex—Many layered, loosely arranged, rounded cells with intercellular spaces in between.

Aerenchyma—Big air-spaces partitioned by one layered partition walls.

Endodermis—One layered, closely arranged, small cells.

Central Cylinder—Vascular bundles are arranged radially. There are 7 groups of vessels and each group having 3 to 6 vessels and few phloem cells arranged in between the xylem groups. Small parenchymatous cells present in between the xylem and phloem cells.

Pith—Composed of parenchymatous cells.

2. L.S. of floating root tip—Fig. 40.

Epidermis—One layered, thin walled cells.

Hypodermis—One layered, parenchymatous cells.

Ground tissue—Many layered, loosely arranged cells with intercellular spaces.

Central Cylinder—Few layered, closely set, slightly elongated cells present. In this meristematic region the vessels have not yet formed.

The aerenchymatous tissues have also not yet developed and instead only the small intercellular spaces are present. The tip is enclosed by thin walled cells which in the absence of root cap protects the meristematic root tip against external injury.

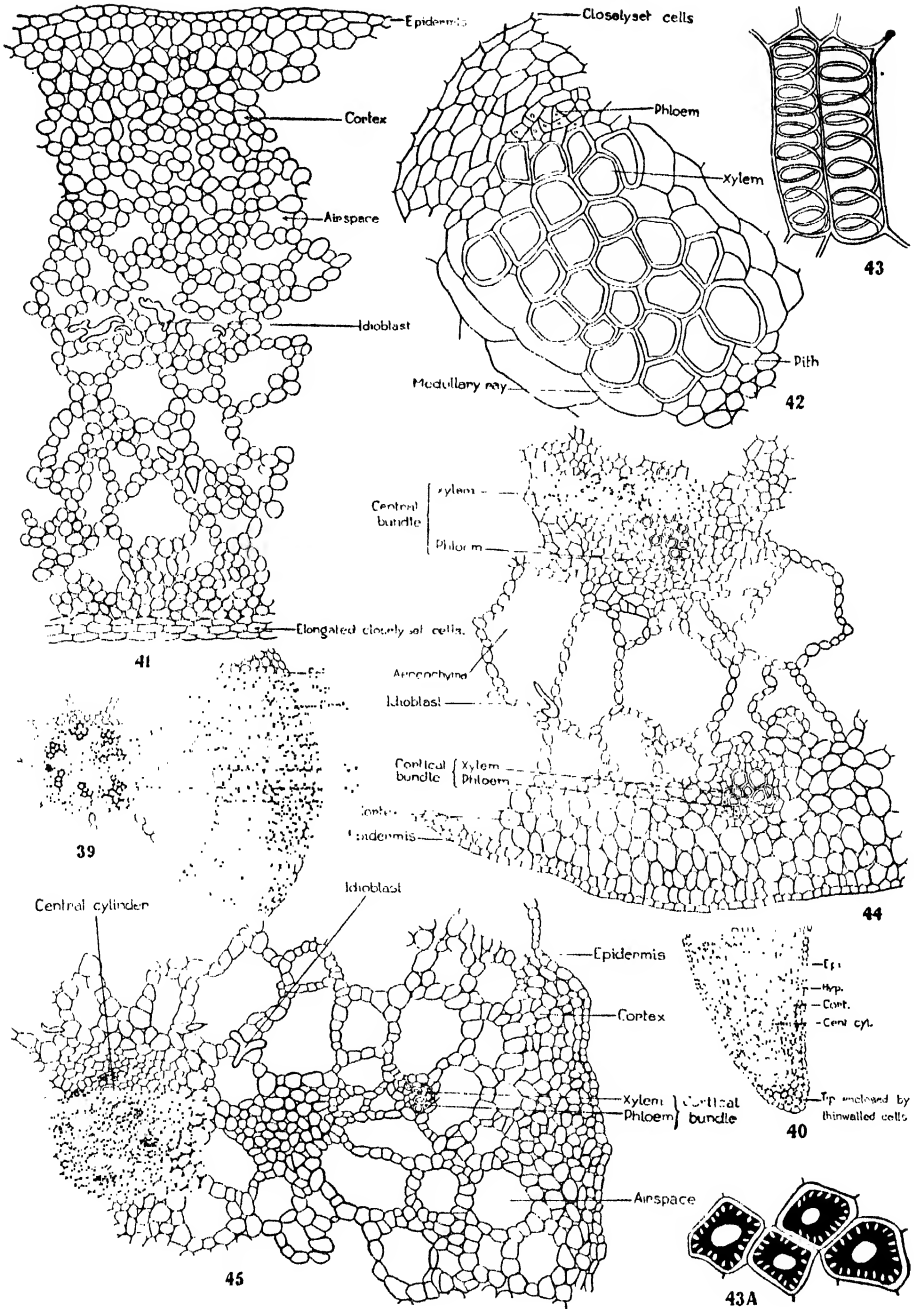
II. TUBER

3. T.S. of underground tuber.

Cortical region—Fig. 41.

Epidermis—One layered, thin walled cells.

Hypodermis—A few layered, closely set, parenchymatous cells.



FIGS. 39—T.S. of floating root ($\times 214$); 40—L.S. of root tip ($\times 214$); 41—T.S. of cortical region of underground tuber ($\times 214$); 42—Central vascular cylinder of underground tuber ($\times 960$); 43, 43A—Vessels of underground tuber ($\times 960$); 43—Spiral thickenings; 43A—Pitted vessels; 44—T.S. of floating tuber ($\times 214$); 45—T.S. of runner ($\times 214$).

Cortex—Many layered, loosely arranged, rounded cells with scattered air-spaces which are larger toward the central region. Branched, thick walled idioblasts projecting in the intercellular spaces are present in a scattered manner.

After the cortex 3 to 4 layers of elongated, closely set cells are present.

Central Cylinder—Fig. 42.

The xylem vessels are arranged in groups just below the closely set cells. Few phloem tissues are present above each group of vessels which are either pitted or thickened spirally (Figs. 43 and 43A). The groups of vessels are separated from each other by medullary ray cells.

Pith—Composed of thin walled, rounded cells with minute intercellular spaces.

4. T.S. of floating tuber—Fig. 44.

Epidermis—Single layered, thin walled cells.

Cortex—Thin walled, irregular shaped cells with minute intercellular spaces. Small vascular bundles are scattered in the cortical region. Each bundle is composed of a few vessels with phloem towards the epidermal region and surrounded by small, parenchymatous cells.

Aerenchyma—A layer of aerenchymatous tissue is present. Idioblasts are present in this layer.

Central Cylinder—Vessels are arranged in a scattered manner with phloem towards the epidermal region and small, parenchymatous cells in between and also surrounding them.

In comparison to the underground tuber the floating tuber has more of air-spaces in the form of aerenchyma. The compact arrangement of the cells of the underground tuber gives more of mechanical support that is needed. In the underground tuber the xylem vessels are in groups in the central region only, and each group of vessels has a group of phloem tissue. But in the floating tuber the xylem vessels are scattered in the central region with one group of phloem tissue only and additional bundles are present in the cortical region.

III. RUNNER

5. T.S. of runner—Fig. 45.

Epidermis—One layered, thin walled.

Cortex—Composed of loosely arranged, rounded cells with small air-spaces near the epidermal region and larger ones toward the central cylinder. Small vascular bundles are scattered in the cortical region. Idioblasts are present.

Central Cylinder—(Fig. 46)—Xylem vessels are arranged in 2 strands. Phloem tissues are present on the outer sides of both the strands. Thick walled, parenchymatous cells are present all round and in between the xylem vessels.

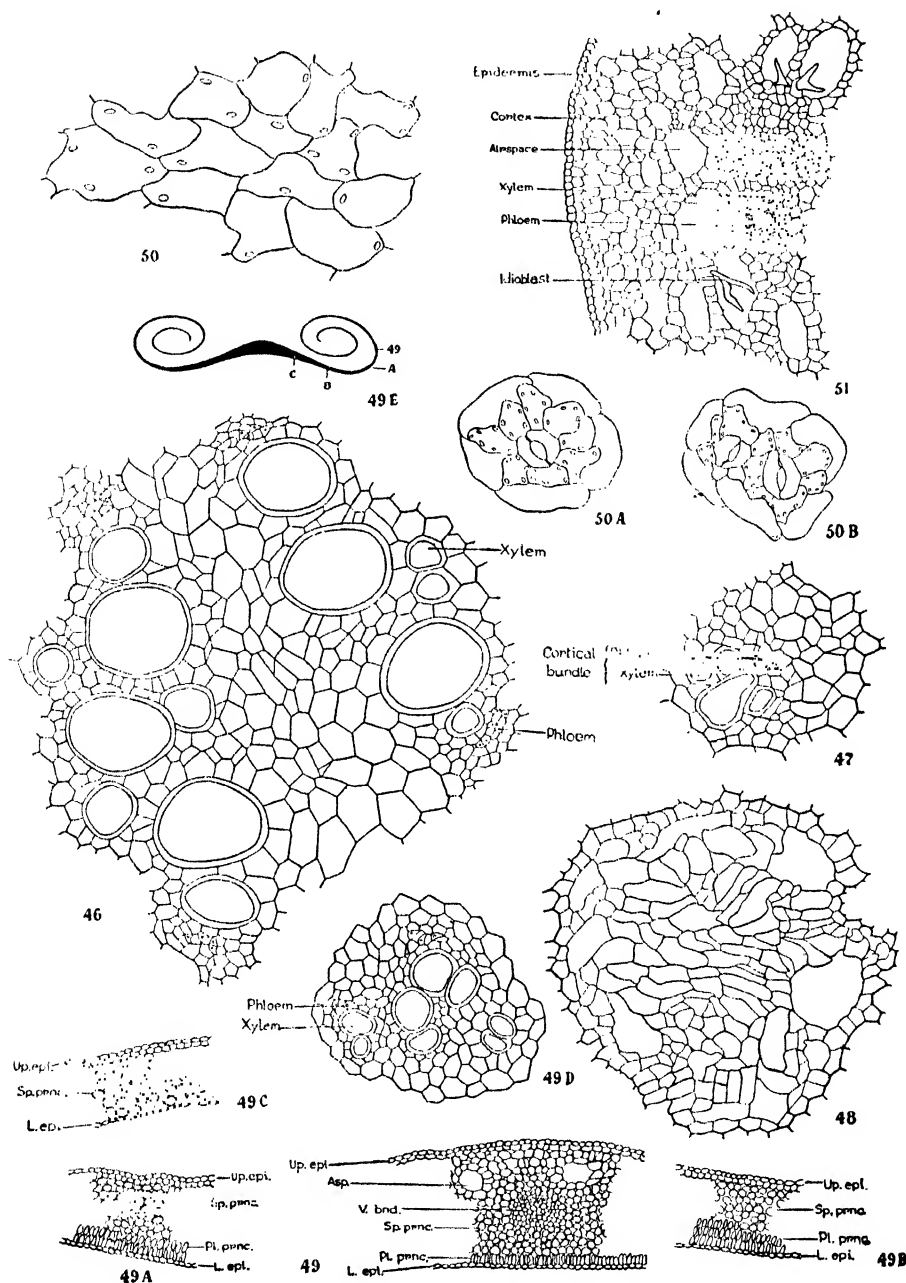
Cortical bundle—(Fig. 47)—Xylem vessels are very few in number and phloem is present only on the outer side of the vessels. The bundle is enclosed by parenchymatous cells.

Diaphragm—(Fig. 48)—The bigger air-spaces are covered with thin walled, cellular trabeculae known as diaphragm.

IV. LEAF

6. T.S. of leaf—Fig. 49.

Epidermis—One layered, thin walled cells on both adaxial and abaxial sides. In aquatic plants the epidermis instead of being protective in



Figs. 46—Central vascular bundle of runner ($\times 960$); **47**—Cortical bundle of runner ($\times 960$); **48**—T.S. of runner showing diaphragm ($\times 214$); **49**—T.S. of leaf with one layer of palisade parenchyma ($\times 214$); **49A**—T.S. of leaf with 2 layers of palisade parenchyma ($\times 214$); **B** = T.S. of leaf with 3 layers of palisade parenchyma ($\times 214$); **C** = T.S. of leaf with palisade parenchyma absent ($\times 214$); **D** = T.S. of leaf with the central vascular bundle ($\times 214$); **E** = A diagrammatic sketch of leaf showing the different regions in it with varying numbers of palisade parenchyma layers; **50**—Lower epidermis of leaf ($\times 960$); **50A**, **50B**—Upper epidermis of leaf ($\times 960$); **51**—T.S. of pedicel ($\times 214$).

function is modified for the absorption of gases and nutrients directly from water and for those functions the surface of the epidermal cells are increased (Fig. 50) by the irregular forms. Stomata are absent from the lower epidermis (Fig. 50) and also the chloroplasts are few in number in individual cells. Stomata are present on the upper epidermis. Each stoma has two guard cells and subsidiary cells varying in number from 7 to 8 and each of them having chloroplasts in them (Figs. 50A and 50B).

Palisade parenchyma—It is either absent or varies in number of layers in different parts of a leaf just next to the lower epidermis. It is absent at the end of a leaf (Fig. 49C). It is present in one layer (Fig. 49) just after that, the number of layers of palisades increasing toward the central region (Fig. 49E) of the leaf. The presence of three layers (Fig. 49B) is much less frequent than the two layers (Fig. 49A).

Spongy parenchyma—Composed of thin walled, rounded cells with small air-spaces, present in between the upper epidermis and palisade parenchyma.

Central Cylinder—Fig. 49D.

Two groups of vessels present in the central region of the leaf with phloem tissue on the upper side of each group. Parenchymatous cells surround the vascular tissue and also present in between them. Vessels are all annular thickened.

V. PETIOLE

T.S. of petiole is same as that of the T.S. of pedicel.

VI. PEDICEL

7. T.S. of pedicel—Fig. 51.

Epidermis—One layered, thin walled.

Hypodermis—One layered, parenchymatous.

Cortex—Many layered, irregular shaped cells with minute intercellular spaces in between. Some big air-spaces are scattered toward the inner region. Idioblasts are present in the air-spaces.

Central Cylinder—Vessels are scattered in the central region with phloem on one side.

As the different vegetative parts of an aquatic plant are all existing in a similar aquatic environment they do not show much of variation in their anatomical structure.

It is already known (Haberlandt, 1914) that the presence of the firm cellulose walls makes it possible for a plant to preserve a constant shape and attain to outward differentiation. The presence of the aerenchymatous tissues makes the plant flexible against the transverse tensions which result when the structure is subjected to bending stresses and thus protects it from being torn or being stripped off bodily. Special stiffening arrangements are particularly necessary in the case of organs which are provided with wide air-spaces. The richly branched, cellular trabeculae that are present within the air passages of aquatic plants are known as diaphragms. In *L. cristatum* few diaphragms are present and in their absence the thick walled, branched or unbranched idioblasts present in the air passages take their place and perform the mechanical function and also prevent flooding of the air passages.

Schwendener (1874) has demonstrated that the species which are restricted to stagnant or slow flowing water (*Potamogeton crispus*, *P. densus*, *P. pectinatus*) developed no specialized mechanical cells either in the cortex or in the central mestome cylinder, the latter itself being able to cope successfully with the very

slight demands that are made upon the tensile strength of the stem. In *L. cristatum* there is complete absence of mechanical tissues and each of the central cylinders is surrounded by closely set, thin walled cells which meet the little mechanical demands made by the vegetative parts.

As shown by Metcalfe and Chalk (1950) the intraxylary phloem is absent and central stele is present with some cortical bundles. The vessels in the underground tuber have pitted and spiral thickenings. The rest of the parts have spiral vessels more commonly.

It is well known that water plants require more ventilating system than terrestrial plants for the need of gaseous exchange. In *L. cristatum* aerenchymatous tissue is found to exist almost throughout the whole body of the plant. These air chambers act as organs for the storage of air and allow ready diffusion of gases within the body of the plant, to facilitate both respiration and photosynthesis. These air-spaces also serve to increase the power of flotation by lowering the specific gravity of the plant body. Reduction in the water conducting strands is very outstanding as the absorption goes on throughout the whole surface of the plant body. As shown by Majumdar (1938) each of the cortical bundles has an endodermis surrounding it without a clearly distinguishable pericycle. He also states that the endodermal cells have well developed casparian strips but the present author has not observed their presence. As shown by Majumdar (1938) the endodermal cells have starch grains in them.

SOME PHYSICO-CHEMICAL CONDITIONS OF EXISTENCE

Lundëgarth (1931) writes that the phenomena one observes in nature are always the resultant of the interaction of a number of more or less independent factors and the investigator must consider these all together. All the factors making up the environment have an influence upon the growth of the species present there, but only in proportion to their relative intensity. Many factors exist together and each of them exerts a direct or an indirect influence on the life of all the vegetation present in a pond. In nature it is almost impossible to find out how a single factor is helping in the growth of a plant. So experiments were carried out in earthenware tubs keeping all factors, except one, constant and then the effect of that one varying factor was found out.

L. cristatum, with the different vegetative and reproductive stages, was collected from several ponds in Calcutta (Bengal), Balasore and Kujang (Orissa).^{*} In Calcutta the ponds were all Corporation tanks and had a depth of approximately 15 to 20 ft. The water in these ponds came from the river Ganges through inlet pipes. The ponds in the other places were all used as fishery nursery tanks and were not open to the public. They had accumulated rain water in them with a depth ranging from 3 to 8 ft. The plants occurred in these tanks almost throughout the year. The physico-chemical characters in some of these tanks, recorded in the course of other investigations, show that the temperature of the water varied from 21.2° to 35.2°C. (time of collection between 10-30 and 11-30 a.m.), pH from 7.3 to 9.2, dissolved oxygen from 2.6 to 17.7 p.p.m., free carbondioxide from nil to 10.56 p.p.m., carbonate from nil to 36.0 p.p.m., bicarbonate from 82.0 to 234.0 p.p.m., nitrate from 0.036 to 1.92 p.p.m., and phosphate from nil to 2.5 p.p.m.

L. indicum, with the different vegetative and reproductive stages, was collected from fishery tanks at Baripada (Mayurbhanj District), Kaushalya Ganga and Nuapara (Puri District) in Orissa. In these tanks the depth of water varied from 4 ft. 6 in. to 12 ft. In these waters the pH varied from 7.3 to 7.8, dissolved oxygen

^{*} Sri S. M. Bannerjee and Sri V. Ramchandran very kindly permitted me to use their unpublished chemical data.

from 6.7 to 8.4 p.p.m., free carbondioxide from trace to 6.0 p.p.m., bicarbonate from 60 to 78 p.p.m., phosphate from 0.12 to 0.21 p.p.m., nitrate from 0.03 to 0.53 p.p.m.

The ponds from which *L. cristatum* and *L. indicum* were collected had the pH ranging from 7.3 to 9.8. Acid waters are seldom found in the places of collection referred to above. It was therefore felt desirable to ascertain experimentally whether these plants could thrive under acid conditions also. Certain experiments on this aspect carried out with *L. cristatum* are briefly reported here.

Experiment I.—In an earthenware tub in which *L. cristatum* was growing healthily, the pH of the water was lowered by the addition of commercial sulphuric acid and it was maintained between 5.5 and 6.5 by the addition of acid frequently. After 15 days the plants showed rotting of root tips and the death of older leaves. The young leaves were still healthy. After another 15 days the roots started reviving and the plants grew as healthily as they grow in alkaline waters. Plants, kept in the control tub in which the pH of the water ranged between 8.7 and 9.0, remained healthy throughout the period of observation.

Experiment II.—In another earthenware tub the pH of the water was maintained between 4.0 and 5.5 by adding acid as in the previous case. On the 6th day the older leaves were half decomposed and the root tips were also affected. From the 10th day majority of the plants were dead and even those that were living had only small, unhealthy leaves which had yellowish green colour. Some of the plants continued to survive in this unhealthy state for a period of two and a half months which was the duration of the experiment. The control tub with the pH ranging between 8.8 and 9.2 had healthy plants all along.

Experiment III.—In another earthenware tub the pH of the water was lowered further with acid and was maintained between 3.0 and 4.0. On the 4th day most of the root tips were decomposed and most of the leaves were half decomposed. On the 9th day the majority of the plants were dead and decomposed and the few rooted plants that survived had unhealthy leaves which were yellowish green in colour and slimy to touch. The control tub which had the pH ranging from 8.7 to 9.3 had healthy plants all along the experiment.

TABLE No. 1

Observations on Limnanthemum cristatum plants growing in aquatic medium of different Hydrogen ion concentration

pH of water	9.0-8.7	6.5-5.5	5.5-4.0	4.0-3.0
After 5 days of the experiment.	Healthy plants.	Healthy plants.	Older leaves half decomposed and root tips also affected.	Most of the root tips and leaves decomposed.
After 15 days.	„	Rotting of root tips and death of older leaves.	Majority of the plants dead and others living in an unhealthy state.	Majority of the plants dead and decomposed and the surviving plants very unhealthy.
After 30 days.	„	Roots started reviving and plants healthy.	Surviving plants in an unhealthy state.	Surviving plants very unhealthy.

It appears from the above observations (Table No. 1) that though the plants grow healthily in fairly alkaline waters, that is, pH ranging between 7.3 and 9.2, they

can tolerate acidic waters up to a pH of 5.5. In more acidic waters (pH between 5.5 and 3.0) only a few plants survive and that also in a very unhealthy state.

In Calcutta (Bengal) most of the ponds are having *L. cristatum* in abundance. In one place within a distance of about 500 yards two ponds are present, one of them having healthy growth of the plant and the other is showing complete absence of it. The pond in which it is completely absent sustains healthy growth of *Vallisneria spiralis* L. The water analyses of the two ponds are as follows:—

TABLE No. 2

Water analyses of the ponds with and without L. cristatum

	Water of the pond in which the plant grows abundantly	Water of the pond in which the plant is absent
pH of the water	7.6	7.8
Dissolved oxygen	14.8 p.p.m.	12.0 p.p.m.
Alkalinity	60 p.p.m.	190 p.p.m.
Phosphate	trace	0.07 p.p.m.
Nitrate	trace	0.32 p.p.m.
Free ammonium	nil	nil
Albuminoid ammonia	9.0 p.p.m.	8.7 p.p.m.
Oxygen consuming capacity ..	14.0 p.p.m.	19.0 p.p.m.

There is no marked difference in the waters of the two ponds, only the pond from where *L. cristatum* is absent alkalinity and nitrate are much more in concentration than where it is present. Observations are now being continued to find out why the plant *L. cristatum* thrives in one pond healthily and is absent from the other.

From another pond in which the plant *L. cristatum* is growing healthily, the bottom mud is collected and after mixing sand with it in different proportion an experiment was set up in earthenware tubs to find out how the plants would grow in different types of substrata (vide Table No. 3).

In nature the growth of plants in an environment is the resultant of the interaction of a number of more or less independent factors. Among all the factors soil is the most important one for a rooted plant. As Lundëgarth (1931) states that from the standpoint of ecology the soil might be defined as that part of the earth's crust which bears life. The rooted water plants, though they are absorbing nutrients from the water even then, obtain a part of their inorganic nutrient from the soil. It is known already that the presence of nutrient salts in the soil furthers the growth of the root system. In pure sand there is lack of nutrient salts and so *L. cristatum* grown on sand did not show healthy growth or survival of the root system. While growing on sand, for a month the plants had quite a good and healthy appearance with flowers but they did not show any further vegetative growth, because when the root system fails to grow healthily the other vegetative parts also do not show healthy growth and development.

TABLE No. 3

L. cristatum grown in earthenware tubs on different types of substrata

Dates	Earthenware Tub 1 Pure pond mud.	Tub 2 Mud—50% Sand—50%	Tub 3 Mud—25% Sand—75%	Tub 4 Mud—75% Sand—25%	Tub 5 Pure sand.
19-8-1954	Ten healthy plants planted in each tub.				
25-8-1954	Plants healthy in all tubs.				
3-9-1954	Plants healthy in all tubs.				
13-9-1954	Good growth	Better growth	Good growth	Better growth	Not very healthy
15-9-1954	Do.	Do.	Do.	Best growth	Unhealthy.
18-9-1954	Do.	Do.	Do.	Do.	Do.
28-9-1954	Healthy but not as in tubs 2 and 4. A few flower buds still present.	Very healthy. Few of the flowers in bloom and a few more buds present.	Healthy with two flower buds but not as healthy as the plants in tubs 2 and 4.	Very healthy. Few of the flowers in bloom and some buds still developing.	Unhealthy. Only a very few small foliage leaves present in an unhealthy state.
After 40 days.	The growth of the vegetative parts is same as the plants in tub No. 2.	The foliage leaves show normal shape and size and very healthy but only the root system is little less branched than those growing in tub No. 4.	The foliage leaves slightly smaller than the leaves on the plants in tubs 2 and 4 and also the root system less branched.	Healthy root system and the other vegetative parts of the plants also very healthy	Only the main root is present without any branching and that is also very thin and delicate. The foliage leaves also are very small in size.

The plant is found to be growing best in a slightly sandy soil, that is, mud 75% and sand 25%. When the substrata was pond mud 50% and sand 50% the growth of the plant was still good but when the proportion of sand increased more than 50% the growth of plants was not as healthy as up to 50%. While growing on pure sand there is neither vegetative nor sexual reproduction and after existing for a month in a healthy condition by using the reserve material as well as the nutrients available from sand and water the condition of the plants started deteriorating. After 40 days the leaves were quite small and the root system was also very scanty (Fig. 38). The plants grown on pure sand might not be dying but they survived in a very unhealthy state and they neither showed vegetative growth nor production of flower buds. Their survival in that unhealthy state might be due to non-availability of the nutrient elements from the substratum. Trough (1953) has stated that the soil acts as the custodian of nutrient elements and the living phase is helped by these elements very much and if a soil was not frugal with its resource of nutrient elements, then a protective vegetative cover would fail to grow. As nutrient elements are absent from sand, the plants growing on sand will show neither healthy growth nor healthy survival.

SUMMARY

1. Experiments have been conducted to verify the observations that *L. cristatum* and *L. indicum* are both rooted aquatics and that they float only for a limited period of their life histories. If by any chance a free floating plant is unable to anchor itself to the substratum within a limited period, it perishes.

2. By studying the external morphology of both the species it is found that the runners come out from that portion of the underground tuber which is jutting out of the soil. The inflorescence develops on the runner a little below the foliage, giving it the appearance of a lateral outgrowth. Each leaf is entire, orbicular or deeply cordate. Floating tuber similarly bears runner, foliage and inflorescence.

3. Both the species reproduce vegetatively and sexually. Vegetative reproduction is commonly carried on by vegetative buds and sexual reproduction by the formation of flowers which ultimately develop seeds. The flowers are in racemose clusters. The flower of *L. cristatum* is pentamerous and of *L. indicum* 5 to 7 merous.

4. Development of inflorescence and flowers of *L. cristatum* has shown that a flower normally takes 33 to 43 days from initial stage up to seed formation.

5. Germination of seed of *L. cristatum* has been worked out. After its formation a seed generally takes 92 days to germinate in tap water.

6. Internal morphology of *L. cristatum* has been studied and it is observed that the vegetative parts do not show much of variation in structure as they are all existing in a similar aquatic environment.

7. Some physico-chemical conditions of existence of *L. cristatum* and *L. indicum* have been studied under natural conditions in detail. It is observed that the tanks in which *L. cristatum* flourished healthily pH varied from 7.3 to 9.2, dissolved oxygen from 2.6 to 17.7 p.p.m., free carbon dioxide from nil to 10.56 p.p.m., carbonate from nil to 36.0 p.p.m., bicarbonate from 82.0 to 234.0 p.p.m., nitrate from 0.036 to 1.92 p.p.m., and phosphate from nil to 2.5 p.p.m. The tanks in which *L. indicum* grew healthily pH varied from 7.3 to 7.8, dissolved oxygen from 6.7 to 8.4 p.p.m., free carbon dioxide from trace to 6.0 p.p.m., bicarbonate from 60 to 78 p.p.m., phosphate from 0.12 to 0.21 p.p.m., and nitrate from 0.03 to 0.53 p.p.m.

Under experimental conditions varying one factor only, such as the pH of the water and the substratum separately, the conditions of existence of *L. cristatum* have been studied. It appears from the observations that though the plants grew healthily in fairly alkaline waters, that is, pH ranging between 7.3 to 9.2, *L. cristatum* can tolerate acidic waters up to a pH of 5.5. In higher acidic waters (pH between 5.5 and 3.0) only a few plants survive and that also in a very unhealthy state. By varying the composition of the substratum it has been found that *L. cristatum* grow best in a slightly sandy soil, that is, mud 75% and sand 25%.

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STUDIES ON GROWTH PRINCIPLES IN LIVER

I. SEPARATION OF CYANO-COBALAMINE FROM OTHER GROWTH FACTORS AND ITS ESTIMATION BY DIFFERENT METHODS

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INTRODUCTION

Recent studies on the active principles of liver indicate that it might contain, amongst others, vitamin B₁₂ complex (Robinson *et al.*, 1952), nucleotides (Hurlbert *et al.*, 1954), nucleosides (Orunesu, 1953), and similar other factors. In 1949 Winsten and Eigen analysed various liver preparations possessing anti-pernicious anaemia or animal protein factor activity, and detected the existence of at least six entities capable of supporting the growth of *L. leichmanii* in the vitamin B₁₂ deficient medium, but these entities have not yet been fully characterised. Shive (1948) suggested the desoxyriboside nature of the alkali-stable bacterial growth factors of liver. Dutta (1955) isolated two such factors and characterised them as a purine and a pyrimidine desoxyriboside.

It was a problem to separate the vitamin B₁₂ in liver homogenates from the alkali-stable growth factors, and to estimate the same. Several authors applied microbiological method (Thompson *et al.*, 1950; Harrison *et al.*, 1951) to estimate vitamin B₁₂ in liver homogenates. Later Boxer and Rickard (1952) devised an efficient method to estimate the total cyanide of the vitamin B₁₂ in liver homogenates. Klaveren *et al.* (1954) reported a method of photometric determination of the vitamin B₁₂, based on the work of Reichstein *et al.* (1951) and Lester and Smith (1952). Rudkin and Taylor (1952) measured the extinction of the purple colour of the dicyano-complex (582 m μ) after having treated the liver extracts for five hours with sodium cyanide at pH 9.5–10.0 and extracting the complex with benzyl alcohol at pH 11–11.5. Lens *et al.* (1952) reported separation of vitamin B₁₂ in liver extracts on aluminium oxide column and estimation by elution and measuring the absorption at 548 m μ .

Liver contains not only cyano-cobalamine but also hydroxo-cobalamine in significant amount. Wijmenga *et al.* (1950) have shown that hydroxo-cobalamine could be easily converted to cyano-cobalamine completely. Exact estimation of the amount of hydroxo-cobalamine in presence of the latter is complex. No microbiological method could serve this purpose, unless they were sharply fractionated into two groups. Boxer and Rickard (1952) suggested the total estimation of cyanide before and after treatment of the substrate with potassium cyanide and thus to calculate the amount of hydroxo-cobalamine. Other colorimetric or photometric method as already referred to could not be modified to serve similarly.

In the present investigation attempt has been made to confirm that besides vitamin B₁₂ there might be present some other growth factors in liver homogenates and subsequently to isolate the cyano-cobalamine from those factors and to estimate it; and lastly to estimate hydroxo-cobalamine in liver extracts.

MATERIALS

- (a) Acetone—purified by distillation in an all-glass distilling set.
- (b) *n*-Butanol—purified by refluxing with pure sodium hydroxide and zinc dust for six hours and then distilling the solvent thrice.
- (c) Pyridine—purified by distillation in an all-glass set.
- (d) Ammonium sulfate (extra pure quality).
- (e) Potassium dihydrogen phosphate (E. Merck).
- (f) Cyano-cobalamine (Merck & Co.).
- (g) Zeo-karb 215 (The Permutit Co., Ltd.).
- (h) Sodium cyanide (extra pure quality).

Beckman spectrophotometer (Model DU) was used for all absorption analyses. Microbiological assays were done with *E. coli* (Bose, 1955)* and with *L. leichmanii* 313 (Robinson *et al.*, 1952).

METHOD

Raw liver was proteolysed with papain (B.P.C.) at 45°C. and the residual undigested protein and proteoses were removed at 0°C. by ethanol in presence of Ca^{++} ion. Clear proteolysed solution had a solid content 20%, total N_2 3% and total α -amino nitrogen 1.9%.

Zeo-karb 215 was used to separate the alkali-stable growth factors from amino acids and the vitamin B_{12} group. The adsorbed vitamin could be eluted with aqueous ammonia and estimated.

A paper chromatographic method was developed to separate the cyano-cobalamine from the other growth principles. Whatman No. 1 paper, impregnated with potassium dihydrogen phosphate, was used. The developing solvent was a mixture of *n*-butanol, pyridine, and water. The cyano-cobalamine was eluted with water and estimated either by microbiological or spectrophotometric method.

In order to have a solution richer in the cyano-cobalamine, the proteolysed solution might be extracted with acetone in presence of ammonium sulfate. After removing the acetone the extract became several times richer in vitamin B_{12} content. When this solution was chromatographed, the separated cyano-cobalamine could be eluted and estimated directly by measuring the absorption at 361 $\text{m}\mu$ (using proper blank eluate).

EXPERIMENTAL

Zeo-karb 215 (3.5 g.) was thoroughly washed with distilled water for about four days and then filled in a glass column (20×0.5 cm.). Then 50 c.c. of dilute hydrochloric acid (3%) was passed (a drop per second) through the column followed by excess of distilled water until the pH of the effluent reached about 6.5. The column was then treated with 1% aqueous ammonia solution (50 c.c.) and rewashed with distilled water until pH of the effluent was about 7.0. The column was re-treated with 3% hydrochloric acid and freed from the acid with distilled water.

One c.c. of standard cyano-cobalamine solution (50 $\mu\text{g.}$ per c.c.) was passed through the column, followed by 9 c.c. of distilled water. Total effluent, 10 c.c.

* The principle of the method is based upon the measurement of the growth zones of test organism after 24 hours of incubation. The assay organisms used are (i) *Lact. leichmanii* 313 and (ii) *Esch. coli* 301 (Mutant). The medium for *L. leichmanii* is a new one prepared here, and that for *E. coli* is one modified from the work of Harrison, Lees and Wood. Variable doses of standard vitamin B_{12} solution and that of test solutions are put on the same agar plate and subsequently the zone diameters are measured. Estimation of vitamin B_{12} is done by finding out the log dose ratio between the curves of the standard vitamin B_{12} and the test solutions, which show a parallel response.

(a), was collected. The column was washed with distilled water, 20 c.c. (b). The column was then eluted with 1.5% aqueous ammonia, 10 c.c. (c). The eluate (c) was concentrated at low temperature (30°C.) to 5 c.c. volume, when ammonia was removed. Absorption at 361 m μ was recorded. The recovery obtained was more than 98% (case 1, vide Table A in 'Discussion').

Studies on hydroxo-cobalamine (its preparation will be described later) with Zeo-karb 215 produced similar results, measuring the E_{\max} at 351 m μ .

It was noted that dilute aqueous ammonia has no deteriorating effect on the cyano-cobalamine or the hydroxo-cobalamine at room temperature (about 30°C.), even if left for several days.

The resin was then regenerated with hydrochloric acid and washed as before.

Two c.c. of proteolysed liver solution, containing 1.5 μ g. vitamin B₁₂ per c.c., was similarly passed through the column, followed by 9 c.c. of distilled water, the effluent being 10 c.c. (d). The column was washed with 30 c.c. of distilled water and the effluent was 30 c.c. (e). The column was then eluted with 1.5% aqueous ammonia, the eluate being 20 c.c., which was concentrated to 2 c.c. (f). Estimation of the vitamin B₁₂ in the fraction (f), according to Boxer and Rickard (1952), gave 2.0 μ g. per c.c., while by microbiological method (Bose) 1.5 μ g. per c.c. (case 2, vide Table A in 'Discussion'). It was noted in several cases that the former method had a tendency to give higher result than the latter method.

Effluent (e) was found to have no growth promoting effect on *L. leichmanii* or *E. coli*. The effluent (d) was a colourless clear solution, showing sharp UV-absorption at 260 m μ and having no absorption from 290 m μ to 400 m μ . It stimulated the growth of *L. leichmanii* 313 and its activity was not reduced by heating at pH 12.0 (110°C./10 min.). Preliminary report of the analysis of this fraction (d) was published elsewhere (Dutta, 1955).

The proteolysed liver solution was then adjuvated so that each c.c. would contain 24 μ g. vitamin B₁₂. One c.c. of this adjuvated solution was similarly analysed as above and found to contain 23.8 μ g. cyano-cobalamine (Robinson *et al.*, 1952) (case 3, vide Table A in 'Discussion').

Paper chromatography was then successfully applied to separate the cyano-cobalamine from other growth factors in liver homogenates. Whatman No. 1 (chromatographic paper) was washed with 1% potassium dihydrogen phosphate solution and dried at 40°C. Two developing solvents were found effective—(I) a mixture of *n*-butanol, ethanol, and distilled water (4:5:2.5), and (II) a mixture of *n*-butanol, pyridine, and distilled water (6:4:3). In the latter case glass distilled water was used. It was noted that the solvent (II) was preferable and so was used for the following investigations. Development was done at room temperature in darkness for six hours.

Experimental work with standard cyano-cobalamine showed that vitamin B₁₂ moved with R_f 0.24 and could be recovered from the paper up to 98%. The alkali-stable growth factors, as isolated by Zeo-karb 215, were also developed on the same chromatogram, when it was noted that they were separated into two groups, one having R_f 0.0 and the other 0.39, and none having R_f 0.24. However, at R_f 0.24 some amino acids seemed to be present after the development of the chromatogram. These amino acids were noted to have no absorption at 361 m μ and no growth stimulating effect on *L. leichmanii* or *E. coli*. All colouring matter remained at the starting zone.

Separation of hydroxo-cobalamine from cyano-cobalamine by the paper chromatogram was also studied. Vitamin B_{12a} (or B_{12b}) was prepared from the cyano-cobalamine thus: an aqueous solution of vitamin B₁₂ was acidified with sulfuric acid to have final concentration 0.001N and final volume 10 c.c. with 20 μ g. vitamin B₁₂ per c.c. It was then exposed to light (two 500 watts projection lamps on both sides of the substrate, which was immersed in a water bath at 25–30°C.) for two hours, when nitrogen gas, purified according to Boxer and Rickard (1952),

was passed through to drive away the liberated CN⁻ groups. The pH of the solution was then adjusted to 6.0 and the absorption spectrum (from 200 m μ to 600 m μ) was recorded to have E_{max} at 351 m μ (instead of 361 m μ) and at 525 m μ (instead of 550 m μ) (Wijmenga *et al.*, 1950). Then aliquots of this vitamin B_{12b} solution was chromatographed, parallel to vitamin B₁₂, and it was noted that the former moved much slower, having R_f 0.05 only, while the latter with R_f 0.24. Thus the cyano-cobalamine can be separated completely from vitamin B_{12b} and the alkali-stable factors.

The cyano-cobalamine, thus separated on the paper, could be eluted with water and then estimated, either microbiologically or by optical absorption at 361 m μ , the latter method requiring a higher concentration of the vitamin (at least 10 μ g. per c.c. of the eluate).

Proteolysed liver solution (containing 2.24 μ g. cyano-cobalamine per c.c.) was chromatographed and the fraction with R_f 0.24 was eluted with water and analysed microbiologically and found to contain 2.10 μ g. cyano-cobalamine per c.c. (case 4, vide Table A in 'Discussion').

A method was developed to extract the vitamin from the proteolysed liver solution and thus to have a concentrated solution, which could be chromatographed, the separated vitamin eluted and estimated by measuring the absorption at 361 m μ . With standard cyano-cobalamine it was noted that from aqueous solution the vitamin could be extracted completely with acetone at 75–80% saturation with ammonium sulfate. Moreover it was noted that acetone, saturated with water and ammonium sulfate, had no significant deteriorating effect on the cyano-cobalamine, even at 100°C. for several hours.

A proteolysed liver solution (50 c.c.), adjuvated so as to contain 20 μ g. cyano-cobalamine per c.c., was mixed with acetone (25 c.c.) and stirred uniformly, when gradually requisite amount of ammonium sulfate was added. It was then centrifuged and the acetone layer collected. The aqueous layer was further extracted twice more with acetone (15 \times 2 c.c.). All the acetone extract was taken together and concentrated at 30°C. to 2.0 c.c. This extract contained some of the alkali-stable growth factors also in addition to the vitamin B₁₂. One c.c. of this solution was treated with Zeo-karb 215 (H-form) and the UV-absorption spectrum of the diluted effluent was recorded (A in Fig. 1). The effluent on being chromatographed showed two zones with R_f 0.0 and 0.39 and both fractions stimulated *L. leichmanii* 313. Then 0.25 c.c. of the above extract was directly chromatographed and the zone with R_f 0.24, parallel to standard vitamin B₁₂, was cut out, eluted with 5 c.c. of distilled water, centrifuged, and its absorption at 361 m μ recorded and each c.c. of the eluate was found to contain 24.2 μ g. cyano-cobalamine. The recovery was 121.0 μ g. in place of 125.0 μ g. (case 5, vide Table A in 'Discussion'). The zones with R_f 0.0 and 0.39 on the chromatogram were detected to contain some alkali-stable growth factors.

The aqueous layer, after the acetone extract, was then passed through a column of Zeo-karb 215 (H-form) and the colourless effluent was collected. The pH of the effluent was adjusted to 7.0 with ammonia when flocculent precipitate appeared. It was centrifuged and the clear solution was analysed. Its UV-absorption curve was recorded (B in Fig. 1), it was chromatographed to note its R_f as 0.0 and this fraction had a stimulating effect on *L. leichmanii* like vitamin B₁₂. The flocculent precipitate was washed twice with water and dissolved in 0.1 N hydrochloric acid and its UV-absorption curve was recorded (C in Fig. 1). It had no bacterial growth promoting property.

Attempt was then made to have an idea about the amount of hydroxo-cobalamine that might be present in proteolysed liver solutions. Proteolysed liver solution containing 1.75 μ g. vitamin B₁₂ per c.c., as estimated by separation on paper chromatogram and assaying by microbiological method, was then treated with sodium cyanide (0.1%), the pH being adjusted to 6.0, and ampouled and

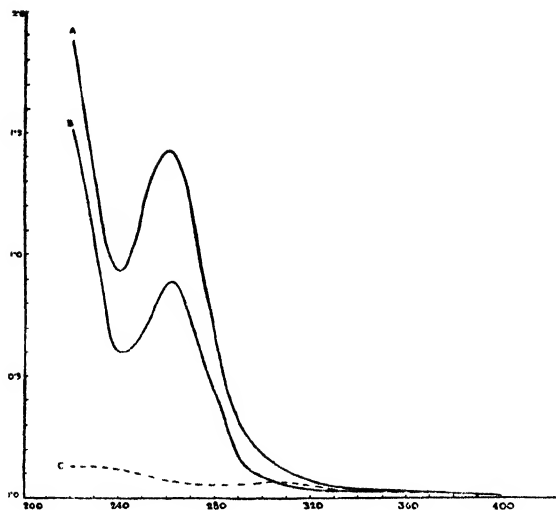


FIG. 1

heated at 52°C. for three hours in darkness and then left in darkness at room temperature for 24 hours. Then 0.08 c.c. of that solution was chromatographed, as before, in darkness and assayed microbiologically (*E. coli.*, Bose) and was found to contain 0.27 μg . cyano-cobalamine. Thus each c.c. seemed to contain 3.37 μg . vitamin B₁₂ in place of 1.75 μg . So 1.62 μg . cyano-cobalamine per c.c. was freshly formed on cyanide treatment of the proteolysed solution.

RESULT

Proteolysed liver solution contained, in addition to the vitamin B₁₂, some vitamin B_{12b} and some alkali-stable growth factors, and the vitamin B₁₂ could be separated from those factors and estimated.

DISCUSSION

Several authors have used various methods to estimate the vitamin B₁₂ in liver extracts. Direct microbiological method might not offer reliable results owing to the presence of other growth factors, like hydroxo-cobalamine and alkali-stable growth factors. The estimation of CN- group according to Boxer and Rickard (1952) gave fair results, but it was a rather long and tedious process. The column chromatographic method of Lens *et al.* (1952) could be applied to only those solutions which contained at least 100–500 μg . vitamin B₁₂ per c.c. and moreover the method became complex for the stringency on the quality of aluminium oxide. Furthermore it was not applicable to purified liver preparation. The combination of paper chromatography with either microbiological or spectrophotometrical method as shown in the present paper is rather simple and requires no special equipments. By this method liver extracts containing as little as 1.2 μg . cyano-cobalamine or hydroxo-cobalamine per c.c. may be accurately assayed. Moreover the degree of purification of the liver extract does not affect the method in any way.

To study the growth principles in liver by the above method, the liver should be proteolysed and freed from undigested proteins and proteoses under mild

conditions, controlling pH, temperature, etc. and giving due attention to the intracellular enzymes, so that the active principles may not be degraded during the preparation of the solution. Concentration of the various fractions was done by vacuum distillation. But freeze-drying is preferable in such cases.

A fairly good recovery of the cyano-cobalamine as shown in this paper would be evident from the results as tabulated below. The results are from an average of five experiments in each case.

TABLE A

Case	Theoretical content of B ₁₂ (μg.)	Found B ₁₂ (μg.)	Method	Ref.
1	50.0	48.0	Spectrophotometric	Boxer and Rickard (1952). Bose (1955) Robinson <i>et al.</i> (1952) Bose (1955)
2	1.5	2.0	CN estimation	
3	24.0	1.5	Microbiological	
4	2.24	23.8	do.	
5	125.0	2.10	do.	
		121.0	Spectrophotometric	

From the above observations it appears that the presence of a stabler form of vitamin B₁₂ as noted by Ramsarma and Shenoy (1955) needs re-examination. Studies on the conversion of the allied substances of vitamin B₁₂ with sodium cyanide and their chromatographic separation and subsequent estimation clearly indicate the presence of vitamin B₁₂ in the proteolysed liver solution.

SUMMARY

Raw liver was proteolysed with papain and freed from undigested proteins, etc. It was then treated with activated Zero-kurb 215 to separate the vitamin B₁₂ from the alkali-stable growth factors. The vitamin B₁₂ group was eluted and assayed microbiologically. Paper chromatography was then applied to separate the cyano-cobalamine from hydroxo-cobalamine and the alkali-stable growth factors. The cyano-cobalamine could be eluted from the paper and estimated either by microbiological method or by spectrophotometry. It was also possible to estimate the amount of hydroxo-cobalamine in the proteolysed solution.

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A STUDY ON PROTEIN HYDROLYSATE WITH REFERENCE TO HISTAMINE AND HISTAMINE-LIKE SUBSTANCES. PART I. EFFECT ON DIFFERENT BIOLOGICAL SYSTEMS

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(Communicated by U. P. Basu, F.N.I.)

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held at Bombay, 6-7 August, 1954)

In the course of antigenicity tests of different batches of protein hydrolysate according to Dale's original technique it was observed in our laboratory that isolated non-sensitized normal virgin guinea-pig's uterus frequently contracts when brought in contact with protein hydrolysate of meat. Considering that the plain muscles of guinea-pig uterus also respond to histamine, a substance likely to be liberated from the degradation of tissues, it was considered worth while to study in detail the reaction brought about by protein hydrolysate in different biological systems in comparison with histamine.

EXPERIMENTAL

Action on uterus.—This was studied both on isolated virgin guinea-pig uterus and rat uterus. The uteri were mounted in a 50 c.c. organ bath containing oxygenated Ringer-Locke solution. The temperature of the bath was kept at 37.5°C. for guinea-pig uterus, and at 32°C. for rat uterus. Doses were given at regular intervals of time.

Action on isolated pieces of guinea-pig ileum.—The intestines were mounted in a 10 c.c. organ bath containing Tyrode solution at 38°C. For neutralization of choline-esters, atropinization was done at a concentration of 10^{-7} , each time before a test substance was put into the bath. For histamine inactivation pyribenzamine 5–10 μ g. was used. Variation of calcium concentration was made, whenever necessary, to test the response at different concentrations of calcium.

Blood pressure in the cat.—For circulatory studies, chloralosed cats were put up (80 mg. per kg. intramuscularly). Comparison of the responses has been made with pure histamine diphosphate. For noting the effect of transfusion a dose of 10 c.c. per kg. was used, the rate of infusion being kept constant at 0.5 c.c. per minute. For detection of vasodepressor action, small doses were injected rapidly.

Protein hydrolysates from meat used for the study were prepared in three batches, carefully following the instructions given in the Indian Pharmacopoeial List (1946). Two batches of casein acid hydrolysate, similarly prepared, were also studied.

RESULTS

Action on guinea-pig uterus.—The tests performed on isolated virgin guinea-pig uteri showed a high order of muscle-stimulating activity of protein hydrolysate. In some experiments even values similar to 10 μ g. of histamine per c.c. were obtained (Fig. I). The presence of such a high quantity was considered alarming particularly when a chemical assay showed the presence of considerable quantity of substances in protein hydrolysate giving Pauly diazo reaction like histamine (Ganguly, 1954), varying from 10–14 μ g. per c.c.

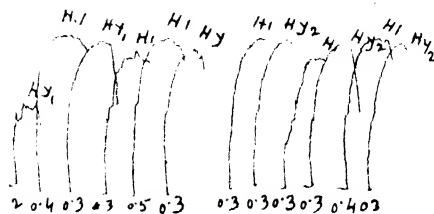


FIG. I

Guinea-pig uterus showing comparison of protein hydrolysate with histamine.

HI = Histamine solution (10 $\mu\text{g./c.c.}$).

Hy = Protein hydrolysate (two samples).

Action on blood pressure.—In order to test whether the stimulating factors had any depressor effect, experiments were put up with chloralosed cats for intravenous transfusion. It was found that rapid intravenous injections of small quantities of protein hydrolysate produced a depressor response similar to that given by histamine, which, though not so high as that shown by the guinea-pig uterus method, was still significant (Fig. II). Considering that a protein hydrolysate containing

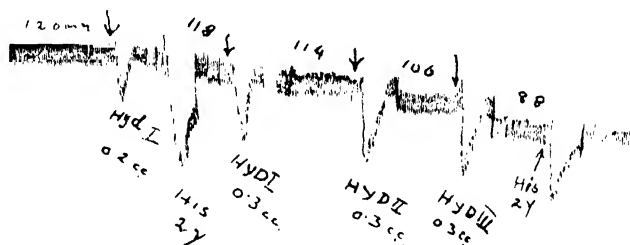


FIG. II

Chloralosed cat (male), 2.7 kg., showing the depressor activity of three batches of protein hydrolysate (meat).

His = Histamine.

Hyd = Protein hydrolysate.

such a high amount of histamine-like substance might give rise to a transfusion shock, a preparation of protein hydrolysate, which had shown previous stimulation of guinea-pig uterus and depressor response in cat, was transfused in large quantity (10 c.c. per kg.) and at a slow rate into an adult anaesthetized cat with normal blood pressure. Instead of a fall of blood pressure, the transfusion was without effect on either the blood pressure or the rate of heart beat. Histamine in dose of 2 $\mu\text{g.}$ was however enough to lower the blood pressure. Further transfusions made on cats after lowering the blood pressure by repeated injections of histamine and/or by carbachol showed a satisfactory recuperation of the hypotensive state. Instead of any vasomotor shock, the blood pressure of the animal began to increase slowly and the heart, which was profoundly depressed by the histamine or carbachol injections, recovered its tone and began beating more forcefully (Fig. III). Transfusion of histamine diphosphate solution (5 $\mu\text{g./c.c.}$) and that of protein hydrolysate containing added histamine (5 $\mu\text{g./c.c.}$) however caused a gradual fall of blood pressure and a steady lowering of vasomotor tone. A transfusion of histamine solution (0.5 $\mu\text{g./c.c.}$) or of protein hydrolysate containing 0.5 $\mu\text{g./c.c.}$ of histamine was without any significant effect.

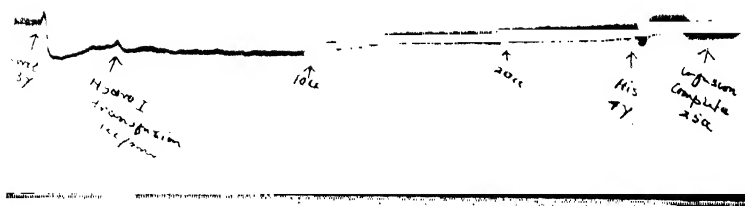


FIG. III.

Cat I. Chloralosed (male).

Shows the increase in vasomotor tone with transfusion of protein hydrolysate after carbachol depression.

Carb = Carbachol.

Action on isolated ileum.—The results of the experiments on cat blood pressure made it necessary to determine the histamine content of protein hydrolysate on isolated guinea-pig's ileum, as this method is considered more specific for histamine. The ileum was suspended in various modifications of Tyrode solution, with normal or diminished concentration of calcium, with or without atropine and/or pyribenzamine. In several batches the spasmogenic activity as detected without atropinization was found to vary from 0.1 to 0.5 μ g. of histamine per c.c. It is significant to note that casein hydrolysate also showed similar presence of histamine-like substances. Since liberation of acetylcholine might be a factor in causing contraction of non-atropinized ileum, the presence of histamine was further checked by full atropinization of the ileum preparation, which became non-sensitive to carbachol, but responded to both histamine and protein hydrolysate (Fig. IV). Pyribenzamine

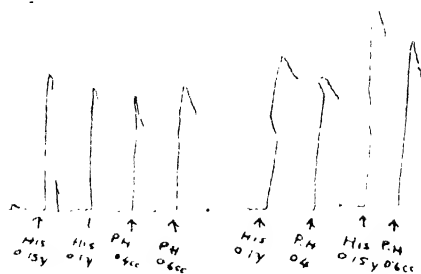


FIG. IV

Atropinized guinea-pig ileum in Tyrode solution.

His = Histamine.

P.H. = Protein hydrolysate.

in 5–10 μ g. doses was able to neutralize fully the effect of both histamine and protein hydrolysate (Fig. V). Considering the known biological behaviour of histamine, it would be justifiable to infer from these experiments that the muscle-stimulating factor in protein hydrolysate, however small it may be, is likely to be

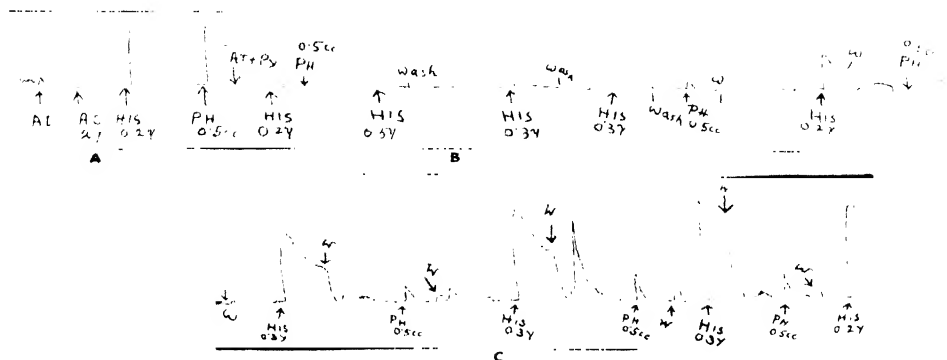


FIG. V

Atropinized (10^{-7}) guinea-pig ileum. A shows the antagonism of acetylcholine (AC) by atropine (At), but non-antagonism of histamine (His) action. B and C are continuous records, showing the differential return of response to histamine and protein hydrolysate, after contact with atropine + pyribenzamine (AT + Py).

W = Wash.

similar to histamine. But the results of work with ileum mounted in low-calcium Tyrode do not warrant such a sweeping conclusion (*vide infra*).

Isolated ileum in low-calcium Tyrode.—It is known that lowering the calcium content of Tyrode makes the ileum less sensitive to stimulation (Gaddum, Peart, and Vogt, 1949; Dalglish, Toh, and Work, 1953). Further experiments with $\frac{1}{2}$ calcium and $\frac{1}{4}$ calcium showed that preparations of protein hydrolysate, which contained originally a definite amount of histamine-like substance in comparison with histamine (not less than $0.2 \mu\text{g}$. per c.c.), was showing a very low response in calcium-deficient medium (Fig. VI). It appears likely from these experiments

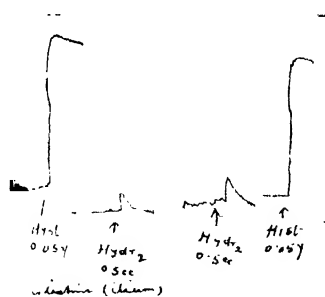


FIG. VI

Guinea-pig ileum in Tyrode with $\frac{1}{2}$ calcium. Shows negligible activity of protein hydrolysate (Hydr).

Hist = Histamine.

that most of the contents of the plain muscle-stimulating factors present in protein hydrolysate might be different from true histamine.

This suggestion of qualitative difference of the histamine-like substances from histamine also receives additional support from the observation with pyribenzamine. It is found that after contact with a mixture of atropine and pyribenzamine for some time and then washing out, the sensitiveness of the ileum preparation returns towards histamine more rapidly than that towards the histamine-like

substances of protein hydrolysate (Fig. V), though both were given in equivalent or near equivalent dosage in term of histamine.

Results on rat uterus.—Rat uterus has been reported by Gaddum to be non-responsive to histamine (Gaddum and Hameed, 1954). Experiments were, therefore, put up with rat uterus on the hypothesis that if the plain muscle-stimulating factor of protein hydrolysate be histamine, it will fail to stimulate the rat uterus. Contrary to this expectation it was found that while histamine showed negligible stimulation of the rat uterus, several batches of protein hydrolysate indicated the presence of factors causing powerful stimulation of the rat uterus (Fig. VII).

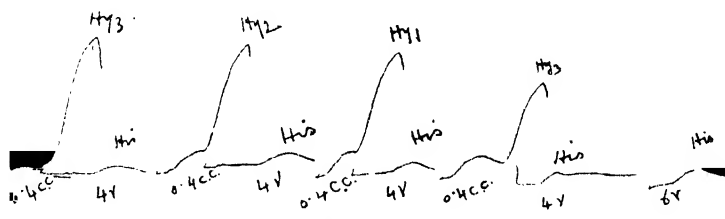


FIG. VII

Rat uterus in Ringer-Locke solution. Shows the selective stimulating property of three batches protein hydrolysate (Hy) compared to histamine (His).

Figures His in μg .
Hy in c.c.

This finding also suggests that the plain muscle-stimulating properties of protein hydrolysate, particularly the factors which stimulate the uterine muscles, may be due to substances other than histamine itself.

DISCUSSION

From the evidence so far presented, it appears reasonable to assume the presence of some definite plain muscle-stimulating factors in protein hydrolysate in fairly large quantities. Though the guinea-pig ileum method suggests that a part of such activity might be due to histamine, yet it could neither account for the high spasmogenic activity on both guinea-pig and rat uterus, nor could it explain the vasodepression with small doses. Infusion of small quantities at a rapid rate to anaesthetized cats with high blood pressure, shows a typical depressor response like a large dose of histamine; but that the product does not really contain histamine to a significant amount can be judged by observing the results of the transfusion of a large amount of the same substance. The question of explaining the vasodepressor action of small doses of protein hydrolysate will, therefore, have to be related to factors other than histamine. In a muscle extract, various metabolites are present which can effect capillary dilatation. From large scale transfusion experiments it also appears reasonable to infer that histamine even if it is present in traces as low as 0.1–0.5 μg . per c.c. is likely to be tolerated in any transfusion of protein hydrolysate.

But the main question, arising out of the findings of these experiments, is related to the nature of the plain muscle-stimulating factors present in protein hydrolysate, particularly the factor causing stimulation of rat uterus and the differential behaviour towards pyribenzamine. Apart from histamine, a plain muscle can be stimulated by other tissue metabolites, such as acetylcholine, adenosine triphosphate, creatin phosphate, adenylic acid, inosinic acid, tryptamine or 5-hydroxytryptamine. It remains to be seen which of these factors will remain stable in an

hydrolysate of muscle protein, after the drastic treatments required for its preparation. A recent paper (Gaddum and Hameed, 1954) has shown that 5-hydroxy-tryptamine causes contractions of both the rat uterus and the guinea-pig uterus. The behaviour of protein hydrolysate towards the rat uterus, as noted in this paper, suggests similarity of action with that of 5-hydroxy-tryptamine. Further confirmation may be had if the activity is found to be neutralized by gramine (3-dimethyl-amino-methyl indole) which acts as a specific antagonist of 5-hydroxy-tryptamine (Gaddum and Hameed, 1954).

It thus appears that further work is necessary to characterize the plain muscle-stimulating factors liberated during protein hydrolysis. Whether done by acid or enzymic digestion it is quite possible that, in a carefully prepared protein hydrolysate from meat, these factors would be found predominant as stimulants of plain muscle than the much-feared substance, histamine. Whether these are connected in any way to adenosine which has vasodilator properties (Sexton, 1953) and adenylic acid system, or tryptamine and 5-hydroxy-tryptamine will have to be worked out. Work on these lines is in progress.

SUMMARY

1. Hydrolysate of protein, particularly from muscle, contains considerable quantity of substances, which stimulate the plain muscles of the uterus and the intestine.

2. Rapid injections of very small doses of such hydrolysate produce a transient vaso-depression similar to histamine. Transfusion of large quantities to both normotensive and hypotensive cats however show no vasodepressive effect. On the contrary the maintenance of a slow and steady rising tone of the vasomotor system proves that majority of these factors are probably not histamine.

3. Experiments on atropinized guinea-pig ileum preparation suggest the presence of histamine in small quantity, which is neutralizable with an antihistaminic. But experiments with Tyrode solution of low-calcium content suggest that the majority of the histamine-like substances, as detected by the guinea-pig's ileum suspended in normal Tyrode solution, is probably not histamine.

4. Rat's uterus responds powerfully to protein hydrolysate, but not to histamine, thus showing that the plain muscle-stimulating factors are probably not histamine but may be similar to 5-hydroxy-tryptamine, or adenosine, or adenylic acid.

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PHOTOPERIODISM IN RICE

VIII. EFFECTS OF SHORT PHOTOPERIOD ON FOUR VARIETIES OF EARLY-WINTER RICE

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INTRODUCTION

Photoperiodic procedures are often adopted by plant breeders to induce simultaneous blooming of varieties that are to be crossed, and to increase the number of generations that can be produced annually and thereby to hasten the breeding of new varieties. Formerly, plant breeders used to experience great difficulties in effecting crosses in a crop like rice, where the flowering dates differ widely and are more or less fixed for individual varieties or strains. These difficulties may be overcome, if the flowering periods are suitably altered by artificially increasing or decreasing the day-lengths.

A knowledge of the photoperiodic requirements of plants may also prove useful to the farmers, when contingencies like flood or drought force them to replant later than the usual time. Since rice occupies an important place in the field crops in our country, the photoperiodic response of many varieties has been studied by different workers (Sircar, 1942, 1946; Sircar and Parija, 1949; Sircar and Sen, 1953; Saran, 1950; Misra, 1954*a*, 1954*b*, 1955*a*, 1955*b*, 1955*c*).

It appears from the available literature that the different rice varieties differ markedly in their photoperiodic responses. The present investigation aims at studying the response of some of the early-winter varieties of rice of Orissa and Uttar Pradesh to short photoperiodic treatments.

MATERIALS AND METHODS

The following varieties were used in the present investigation:

- (i) T.1145 (a selection from Usha of Puri district, Orissa),
- (ii) T.36 (a selection from No. 1 Cuttack, Orissa),
- (iii) T.23 (a selection from Kala Sukhadas of Banda district, U.P.),
- (iv) T.17 (a selection from Bansi of Allahabad district, U.P.).

Pure strain seeds of these varieties of rice were kindly supplied by the Director, Central Rice Research Institute, Cuttack, and the Rice Specialist, Nagina Rice Research Institute, Bijnor, U.P. A 10-hour (8-00 a.m. to 6-00 p.m.) short photoperiodic exposure was given to one-week old seedlings for periods of 3, 4, 5 and 6 weeks respectively in the seed-bed to different experimental lots. Subsequently, the seedlings were transplanted in the field along with the controls grown in the natural period of sunshine. In one of the lots receiving the short-day treatment for 6 weeks in the seed-bed, the treatment continued till panicle emergence.

EXPERIMENTAL RESULTS

Time of heading

Observations regarding the period of ear emergence from the time of sowing are presented in Table I.

TABLE I

Average period from sowing to first panicle emergence (days). Seed sown June 18, 1949; Tests began June 25, 1949. Seedlings transplanted August 7, 1949

Varieties	T.1145	T.23	T.36	T.17
Treatments				
Control	119-55	119-10	119-50	115-90
S. Day for 3 weeks	120-95	124-10	123-90	119-40
S. Day for 4 weeks	123-30	126-60	127-00	122-50
S. Day for 5 weeks	127-80	128-30	129-60	123-00
S. Day for 6 weeks	128-00	131-75	131-90 in 65% 69-20 in 35%	124-90 in 75% 74-00 in 25%
S. Day prolonged till ear emergence	101-80	108-50	80-00 in 50% 65-30 in 50%	99-20 in 70% 68-00 in 30%

An examination of the data in Table I shows that all the four early-winter varieties do not behave alike to short-day treatments. In varieties T.1145 and T.23, the short-day treatments in seed-bed retard the time of ear emergence. This retardation effect increases with an increase in the duration of short-day treatment in the seed-bed. Contrariwise when the short-day treatment is administered for a period of 6 weeks in the seed-bed and prolonged further after transplantation till heading, the ear emergence is greatly accelerated.

In varieties T.36 and T.17, short-day treatments for 3, 4 and 5 weeks delay the ear emergence. The 6 weeks' seed-bed treatment produces a delaying effect to an extent of 65 per cent of the population in T.36 and 75 per cent in T.17, the rest of the plants depict acceleration effects. The prolonged treatment till ear emergence induces early heading in 100 per cent plants. Here again, two periods of heading are noted, a certain percentage of plants is characterized with greater earliness and the others with less so. In these two varieties, however, the remaining tillers flowered much later than the mains (Table II).

TABLE II

Time of ear emergence of the main shoots and tillers in varieties T.36 and T.17.

Varieties	Average days from sowing to ear emergence			
	T.36		T.17	
	Main shoot	Remaining tillers	Main shoot	Remaining tillers
Treatments				
Control	119-50	122-00	115-90	118-00
S. Day for 6 weeks in seed-bed	69-20	132-14	74-00	126-60
S. Day prolonged till ear emergence	65-30	103-44	68-00	98-66

Flowering in the control plants was over within four days. It appears, thus, that short-day exposure of these varieties results in uneven flowering of the main

shoot and tillers. The tillers of the plants which received the short-day treatment for 6 weeks in the seed-bed in these varieties, invariably flowered a few days later than the corresponding tillers in the control plants. It is interesting to note that tillers of the plants which received short days till ear emergence flowered much earlier than those belonging to the lot which received but 6 weeks' treatment in the seed-bed.

Tillers, leaves and plant height

Records on tiller and leaf number and the height of the plants were taken at regular intervals, four times during the life span of the plants. The following observations are noteworthy:

(a) Short-day treatments for 3, 4, 5 or 6 weeks in the seed-bed produce a beneficial effect on the production of tillers but when prolonged even after transplantation until heading a considerable reduction in tiller production is noticeable, specially towards the later stages, variety T.36 being an exception to this.

(b) The production of leaves is favourably affected by the short-day treatments in the seed-bed stage. Contrariwise, following prolonged short-day treatment, the production of leaves is greatly reduced except in plants of variety T.36.

(c) The first three measurements on height of plants, following short-day treatments of different durations, do not exhibit any material change. The final height is, however, slightly reduced in the lot receiving 6-week seed-bed treatment. In the lot receiving the prolonged treatment, there is an indication of less elongation in earlier measurements and much more so in the final.

Grain yield

When grains had matured fully, the ear heads were collected. The ears were left to dry in the sun for one week and their weights recorded. Such data are shown graphically in figure 1. It is obvious that short-day treatments in the seed-bed for 3 to 6 weeks do not affect the grain yield to any appreciable extent; the prolonged short-day treatment, on the contrary, adversely affects the grain yield in all the four varieties.

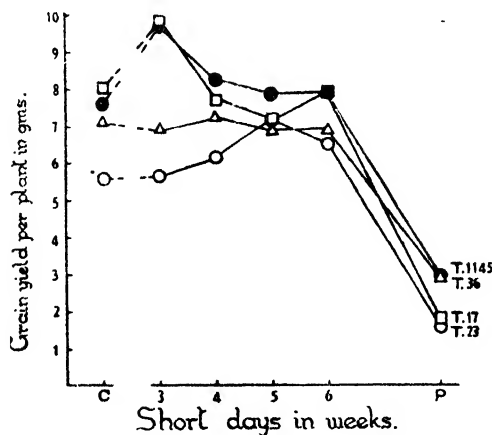


FIG. 1. Grain yield per plant in gms. in four early-winter varieties (T.1145, T.23, T.36, and T.17) of rice when the 7-day old seedlings are subjected to 10-hour short days for 3, 4, 5 and 6 weeks and till ear emergence. The grain yield in prolonged treatment set (P) is considerably less in comparison with the control set (C) receiving full length of day.

Components of yield

The data on components of yield, e.g. number of panicles, length of panicles, number of grains, number of spikelets, percentage of grain set and yield of one thousand grains, are presented in Table III. Statistical analysis of these data is shown in Table IV.

TABLE III
Components of yield: average of 20 plants

Varieties	T.1145	T.23	T.36	T.17	Mean	Per-centage
<i>Treatments</i>	<i>Number of panicles per plant</i>					
Controls	4.00	2.05	2.20	3.30	2.88	100.0
S. Day for 3 weeks	4.20	1.95	2.05	3.10	2.82	97.9
S. Day for 4 weeks	4.30	2.30	2.30	2.95	2.96	102.8
S. Day for 5 weeks	4.95	2.50	2.45	2.80	3.17	110.1
S. Day for 6 weeks	4.20	2.75	2.60	2.95	3.12	108.3
S. Day prolonged till heading	3.00	1.55	2.70	1.75	2.25	78.1
	<i>Length of the panicle in cm.</i>					
Controls	18.40	27.02	23.26	21.32	22.50	100.0
S. Day for 3 weeks	19.02	24.48	24.14	22.54	22.54	100.2
S. Day for 4 weeks	18.14	24.08	23.36	21.16	21.68	96.3
S. Day for 5 weeks	17.32	23.52	23.42	21.98	21.56	95.8
S. Day for 6 weeks	17.50	23.80	22.54	20.06	20.97	93.2
S. Day prolonged till heading	11.96	14.68	11.40	12.44	12.62	56.1
	<i>Number of grains per panicle</i>					
Controls	90.30	112.16	98.16	96.92	99.38	100.0
S. Day for 3 weeks	108.06	117.74	102.46	131.48	114.93	115.6
S. Day for 4 weeks	94.64	111.08	97.34	122.28	106.33	107.0
S. Day for 5 weeks	76.44	119.82	88.22	112.18	99.16	99.8
S. Day for 6 weeks	89.88	100.48	84.02	115.42	97.45	98.0
S. Day prolonged till heading	53.26	44.08	39.72	45.96	45.75	46.0
	<i>Number of spikelets per panicle</i>					
Controls	107.90	138.22	123.32	115.80	121.31	100.0
S. Day for 3 weeks	131.54	145.82	135.42	149.06	140.46	115.8
S. Day for 4 weeks	116.32	136.16	121.48	143.64	129.40	106.7
S. Day for 5 weeks	103.92	146.00	108.54	130.04	122.35	100.8
S. Day for 6 weeks	115.00	120.94	103.14	137.64	119.18	98.2
S. Day prolonged till heading	68.96	55.48	47.36	59.42	57.80	47.6
	<i>Percentage of grains set per panicle</i>					
Controls	83.80	80.94	79.58	83.44	81.94	100.0
S. Day for 3 weeks	82.62	80.02	75.78	88.08	81.62	99.6
S. Day for 4 weeks	81.30	81.54	80.10	85.18	82.03	100.1
S. Day for 5 weeks	73.46	82.44	81.22	85.80	80.73	98.5
S. Day for 6 weeks	78.24	83.10	81.50	84.02	81.71	99.7
S. Day prolonged till heading	77.68	79.42	83.76	77.40	79.56	97.1
	<i>Weight of one thousand grains in gms.</i>					
Controls	20.84	23.92	33.00	25.54	25.82	100.0
S. Day for 3 weeks	20.90	24.74	33.18	24.10	25.73	99.6
S. Day for 4 weeks	20.22	24.20	32.46	22.54	24.85	96.2
S. Day for 5 weeks	20.92	24.60	31.94	23.08	25.13	97.3
S. Day for 6 weeks	20.88	24.54	31.58	23.76	25.19	97.6
S. Day prolonged till heading	19.82	23.34	27.98	23.28	23.60	91.4

TABLE IV

Analysis of variance of the data of components of yield

			<i>Number of panicles per plant</i>					
		D.F.	S.S.	M.S.	F	5%F	1%F	
Varieties	..	3	67.37	22.45	86.34**	2.70	3.98	
Treatments	..	5	11.07	2.21	8.50**	2.30	3.21	
Interaction (V × T)	..	15	12.44	0.83	3.19**	1.77	2.25	
Error	..	96	25.25	0.26				
Total	..	119	116.13					
			<i>Length of panicle</i>					
Varieties	..	3	560.75	186.91	93.92**	2.70	3.98	
Treatments	..	5	1456.45	291.29	146.37**	2.30	3.21	
Interaction (V × T)	..	15	110.79	7.38	3.70**	1.77	2.25	
Error	..	96	191.90	1.99				
Total	..	119	2319.89					
			<i>Number of grains per panicle</i>					
Varieties	..	3	9086.98	3028.98	16.37**	2.70	3.98	
Treatments	..	5	59708.52	11941.70	64.56**	2.30	3.21	
Interaction (V × T)	..	15	6562.30	437.48	2.36**	1.77	2.25	
Error	..	96	17756.80	184.96				
Total	..	119	93114.60					
			<i>Number of spikelets per panicle</i>					
Varieties	..	3	8044.90	2681.63	10.23**	2.70	3.98	
Treatments	..	5	84762.16	16952.43	64.73**	2.30	3.21	
Interaction (V × T)	..	15	8024.01	534.93	2.04	1.77	2.25	
Error	..	96	25142.37	261.89				
Total	..	119	125973.44					
			<i>Percentage of grains set per panicle</i>					
Varieties	..	3	340.54	113.51	6.62**	2.70	3.98	
Treatments	..	5	90.98	18.19	1.06	2.30	3.21	
Interaction (V × T)	..	15	824.62	54.97	3.20**	1.77	2.25	
Error	..	96	1645.27	17.13				
Total	..	119	2901.41					
			<i>One thousand grain weight</i>					
Varieties	..	3	1991.48	663.82	670.52**	2.70	3.98	
Treatments	..	5	64.31	12.86	12.98**	2.30	3.21	
Interaction (V × T)	..	15	67.12	4.47	4.51**	1.77	2.25	
Error	..	96	95.21	0.99				
Total	..	119	2218.12					

**Significant at 1% level.

A careful survey of the data presented in Tables III and IV leads to the following observations:

Number of panicles

Short-day treatment for 3 or 4 weeks in the seed-bed is without any significant effect on panicle formation, but when supplied for 5 and 6 weeks an increase in the

number results. A conspicuous decrease in panicle number is, however, obtained in all the four varieties of plants when short-day treatment is, otherwise, prolonged till ear emergence.

Length of the panicle

The short-day treatment for 3 and 4 weeks is again without any effect on panicle elongation; that for 5 and 6 weeks' duration induces but slight stunting. When this treatment is prolonged till heading time, a marked decrease in the length of the panicle is noticeable.

Number of grains per panicle

The number of grains set per panicle increases as a result of short-day treatment for 3 weeks in the seed-bed. This increase is significant at 5 per cent level. The short-day treatments for 4, 5 and 6 weeks, however, do not induce any marked change in this regard, compared to controls. When the short-day treatment is continued beyond the 6th week stage till ear emergence, a very adverse effect on grain formation is noticed. The decrease in the number of grains formed due to this treatment is very marked and is highly significant.

Number of spikelets per panicle

The number of spikelets assumes a trend similar to the number of grains per panicle. Short-day treatments for 3 weeks initiated an increase in this regard over the controls. No such deviation is recorded following 4, 5 and 6 weeks' treatments. The prolonged short-day treatment, however, produces a very adverse effect on spikelet formation, thus bringing about a significant decrease relative to controls.

Percentage of grains set per panicle

Percentage of grains set per panicle is almost alike in the untreated controls and in lots where short-day treatment in the seed-bed lasted 3, 4, 5 or 6 weeks. It is interesting to note that although the number of grains per panicle formed under the prolonged short-day treatment is greatly reduced, the percentage of grains set per panicle is not adversely affected.

Weight of one thousand grains

Short-day treatments for varying periods in the seed-bed do not initiate any appreciable change in the absolute weight of grains, but when prolonged till heading time, a small decrease is observed in all varieties except T.36 where the decrease is rather considerable.

DISCUSSION

The foregoing results present very interesting data regarding the developmental physiology of the rice plant. The photoperiodic responses of four early-winter varieties of rice, two procured from Orissa and two from U.P., exhibit varied tendencies. There is a gradual delay in panicle emergence in the two varieties T.1145 and T.23 as a result of short-day exposures for 3 to 6 weeks in the seed-bed. When, however, the same exposure is prolonged till heading time a marked earliness is noticed in this regard. In the varieties T.36 and T.17, short-day treatment for 3, 4 and 5 weeks in the seed-bed similarly delays panicle emergence, but six weeks' exposure induces an earliness in certain cases and delay in others. When the treatment is prolonged till heading time, panicle emergence in varieties T.36 and T.17

occurs in two instalments, first being characterized with earliness than the other. From the standpoint of photoperiodic sensitivity, it is clear that varieties T.36 and T.17 are more sensitive than T.1145 and T.23. These former ones show earliness of heading in a certain percentage of plants following a treatment of 6 weeks in the seed-bed and a 100 per cent earliness in the prolonged treatment lot.

The peculiar response of some plants exhibiting early heading, when subjected to short-day treatment, as opposed to a differential response by others of the same lot, may better be explained on a more detailed enquiry in the field. In a study of the photoperiodic induction and development of the growing apex in Rupsail, a winter variety of rice, Sircar and Sen (1953) similarly recorded that following 2 weeks of short-day treatment in the seed-bed only 3 per cent, following 3 weeks 66 per cent and following 4 weeks 100 per cent of the population of plants showed flowering of the main shoot. Anatomical changes in the growing apices reveal that, after 3 weeks, some of the vegetative apices become reproductive while others remain vegetative. After 4 weeks all the apices become reproductive. It may be because photoperiodic stimulus is not likely to be uniformly perceived by all the plants under a particular treatment. It is also possible that all the plants are not at the same stage of development to receive the stimulus conducive to initiate earliness in heading.

It was also observed that in plants where the main shoots showed early ear emergence, the remaining tillers flowered about the same time as the controls or even later. Such uneven flowering of the tillers and the main shoot had been observed by Kondo, Okamura, Isshiki and Kashahara (1932) in some Japanese varieties, by Beachell (1943) in American varieties and by Sircar and Parija (1949) in Indian varieties of rice. The two different periods of heading were, apparently, due to the fact that no tillering had occurred by the time the seed-bed treatments had concluded. It appears that the photoperiodic perception for a period of 5 or 6 weeks, inducing early flowering, is limited to the main shoot, while the tillers remain unaffected. According to Sircar and Parija (1949), the floral response of the main shoot and tillers may be related to the distribution of the flower forming substance in the growing region. Hormone synthesized in the leaves exposed to short days is thus, presumably, first translocated to the growing points of the main shoot and induces flowering, while the tiller buds formed after the period of exposure receive, obviously, but little quantity of the hormone, and consequently the flowering in tillers is late as in the controls. This explanation finds favour in the work of Cailahjan (1936) with *Chrysanthemum*, hemp and *Perilla*, where failure of flowering was observed due to the movement of the flowering hormone in insufficient quantity. Harder, Witsch and Bode (1942) also found suppressed flowering even changing in various degrees to vegetative structures, due evidently to variations in the supply of the flower-forming stimulus. A similar effect was noted in the soybean by Borthwick and Parker (1939). According to Murneek (1939) flower production may be suppressed or their development may be incomplete when there is a lack of sufficient quantity of the hormone.

This differential behaviour of the main axis with the tillers in regard to flowering is, however, not seen in Petkus winter rye in low-temperature vernalisation treatment (Purvis, 1940). She viewed that the effective substance increases after the termination of the low-temperature treatment, for even all the secondary branches formed later are also vernalised, though never exposed to low temperature. Deliberate removal of the main axis and the first crop of tillers, followed by a second removal, also in no way annul the temperature after-effect.

Whatever be the behaviour of tillers, considerable earliness in heading of the main shoot is observed for all the four early-winter varieties of rice as a result of short-day treatment prolonged till ear emergence. Similar observations were made by Yoshii (1926) and Tabata, Tedsuka and Hatabuchi (1934) for Japanese varieties of rice, by Beachell (1943) for American varieties of rice, by Sircar (1942),

Kar and Adhikari (1944-45), Kar (1946), and Sircar and Parija (1949) for Bengal varieties of winter rice, by Saran (1945) for Bihar varieties of winter rice and by Garner and Allard (1920, 1925, 1931) for other crops.

It is noteworthy that although prolonged short-day treatment brings about a significantly earlier heading, the average number of tillers and leaves and plant height are much reduced and this feature is maintained throughout the life period of the plants. Growth retardation following photoperiodic treatments had also been reported by Murneck (1937). He observed complete inhibition of stem development in *Rudbeckia*, partial curtailment of growth in *Soja* and some similar effects on the vegetative organs of *Cosmos* and *Salvia*. He termed this type of growth inhibition as photoperiodic inhibition.

In all the different varieties of rice receiving prolonged short-day treatment, the feature for earlier emergence of heading is associated with a marked decrease in other directions, e.g. in the average number of panicles and grain yield per plant, average length of panicles, and number of grains per panicle. Beachell (1943) obtained similar marked decreases in plant organs of five sensitive American varieties of rice. Working with Japanese varieties of rice, Tabata, Tedsuka and Hatabuchi (1934) observed that short-day treatments of 8 hours per day reduced the yield of grains. Yoshii (1926) reported that daily light periods of 5 and 9 hours, when supplied to late maturing varieties of rice, though increased the number of tillers per plant, reduced the length of panicles, yield of grain and plant height. Sircar and Parija (1949) also noted a reduction in ear length and the number of grains set in Rupsail subjected to 10-hour photoperiod. Sircar (1946), however, recorded a higher grain yield in another less sensitive winter rice (Bhasamanik) following the seed-bed photoperiodic treatment of 8 hours for 4 or 6 weeks or when continued till heading time.

In plants of varieties T.1145 and T.23, varying degrees of delay in ear emergence has been noted consequent to short-day treatments in the seed-bed. In these very varieties, some of these photoperiodic treatments also induce an increase in grain yield. It might prove useful to try these experiments on a large scale in the field. Such experiments are not only likely to furnish confirmatory evidence but may also give an idea of the economic possibilities. These results are in contrast with the findings of Sircar (1946) who obtained an earlier heading in Bhasamanik (a variety of Bengal rice) coupled with increase in grain yield on the application of 8-hour photoperiod for 4 to 6 weeks in the seed-bed.

SUMMARY

In this work, effectiveness of 10-hour short-day (8-00 a.m. to 6-00 p.m.) treatment on four early-winter varieties of rice from Orissa and Uttar Pradesh has been studied in pot culture experiments. These four varieties of rice do not respond uniformly to short days. Short-day treatments in the seed-bed for 3 to 6 weeks retard the time of ear emergence in varieties T.1145 (Usha of Puri district) and T.23 (Kala Sukhadag of Banda district). The retardation effect is found to be more, the longer the duration of the treatment in the seed-bed. Where the short-day treatment is given for 6 weeks in the seed-bed and prolonged till heading, the ear emergence is greatly accelerated. Contrariwise, in the varieties T.36 (a selection from No. 1, Cuttack) and T.17 (Bansi of Allahabad district), seed-bed treatments for 3, 4 and 5 weeks delay the ear emergence. A similar treatment for 6 weeks produces a delaying effect in 65 per cent plants of variety T.36 and in 75 per cent of variety T.17. The rest of the plants are characterized with acceleration effects. In the lot subjected to prolonged short-day treatment, earliness in ear emergence is observed in 100 per cent plants. Here again, two periods of heading are noted, a larger percentage of plants exhibiting greater earliness and a smaller one showing less so. On confirmation by large scale trials in the field, some of these findings may prove of economic significance.

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STUDIES ON THE MECHANISM OF THE DIABETOGENIC ACTION OF ALLOXAN

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INTRODUCTION

Alloxan produces diabetes in a large number of species of animals by a selective destruction of the beta cells of the islets of Langerhans. The diabetogenic action of alloxan is exerted directly on the islet cells and almost immediately after it reaches the blood, the degranulation and degeneration of the beta cells being the ultimate result of this initial action (Lukens, 1948). The mechanism of this initial action of alloxan has not been adequately explained (Lukens, 1948; Lazarow, 1949, 1954).

Diabetogenic doses of alloxan destroy only the beta cells and leave other tissues, including the alpha and the acinar cells of the pancreas itself, unaffected. Thus, the action of alloxan is highly selective. It is, however, not specific for the beta cells—large doses of alloxan cause renal and hepatic necrosis as well (Dunn and McLetchie, 1943; Goldner and Gomori, 1943; Lukens, 1948).

The remarkable selectivity of alloxan for the beta cells possibly excludes any general cytotoxic mechanism in the development of alloxan diabetes. It, therefore, seems reasonable to suppose that the fundamental action of alloxan consists of interference with some essential enzyme or enzymes of the beta cells. Lack of any specificity of alloxan for the beta cells, however, suggests that the enzyme(s) concerned is not restricted to the beta cells, and is not, therefore, one of those specialized enzymes involved in the synthesis of insulin. The enzyme is presumably present in other tissues, e.g. the kidney, which are also affected by suitable concentrations of alloxan, the selectivity of alloxan for the beta cells possibly arising as a result of the metabolic specialization of these cells. As has been pointed out by Lazarow (1954), this metabolic specialization may be associated with (a) a low concentration of the enzyme concerned within the beta cells, (b) a lessened ability of the beta cells to destroy or detoxify alloxan, or (c) a diminished concentration of a compound which protects the enzyme against inhibition by alloxan.

Protection against alloxan diabetes by glucose, mannose, or fructose, but not by any other sugar or sugar-derivative (Sen and Bhattacharya, 1952; Bhattacharya, 1953, 1954) lent considerable support to the enzyme-inhibition theory of alloxan action, and also offered an explanation of the selectivity of alloxan for the beta cells. The present paper reports the results of *in vitro* studies on the effect of alloxan on a number of enzymes concerned with the utilization of glucose. The primary object of the experiments described here was to find out whether the antagonism between the hexoses and alloxan with respect to the development of diabetes in animals could be simulated *in vitro* with respect to any enzyme or enzyme system which might be considered essential for the life of the cells.

EXPERIMENTAL RESULTS

Materials

Succinic dehydrogenase and cytochrome oxidase.—Thoroughly washed pigeon breast muscle (Umbreit *et al.*, 1947) was used as the source of both of these enzymes. The washed muscle (1 gm.) was ground with sand and 9 ml. 0.1 M phosphate buffer, pH 7.4 in the cold for 30 minutes. The suspension was centrifuged at 3,000 r.p.m. for 10 minutes and the supernatant used for the experiments. It showed no blank O_2 uptake.

Cytochrome c.—Cytochrome c was prepared from pigeon breast muscle by the method of Keilin and Hartree (1937) and standardized spectrophotometrically.

Pyruvic oxidase.—Pigeon 'brain dispersion' prepared according to Peters and Wakelin (1946) was used in the tests.

Triosephosphate dehydrogenase.—(a) Dialyzed extract of acetone-dried rabbit muscle powder (Green, Needham and Dewan, 1937) was used as the source of the enzyme. (b) *Cozyme-free triosephosphate dehydrogenase* was obtained by gently shaking the dialyzed extract (a) with norit (500 mg./10 ml.) in the cold and removing the norit by centrifugation and filtration (Rapkine, 1938; Dickens, 1946). Two treatments made the enzyme almost completely free from cozymase.

Lactic dehydrogenase and malic dehydrogenase.—These enzymes were also studied in the dialyzed extract of acetone-dried rabbit muscle powder (Green *et al.*, 1937; Dickens, 1946). The dialyzate was diluted five times for use.

Flavoprotein (Diaphorase).—This enzyme was prepared from beef heart muscle by the method of Straub (1939). The purification was carried as far as the elution from C_7 alumina.

Catalase.—Rat liver was sliced and washed thoroughly to remove as much blood as possible. It was then homogenized in ten volumes of distilled water and kept in ice for 24 hours with a few drops of chloroform. After 24 hours the suspension was centrifuged and the supernatant, diluted about 200-fold, was used as the source of the enzyme.

D-amino-acid oxidase.—Following the method of Mann and Quastel (1946) fresh rat kidneys were ground with sand and ten times their weight of distilled water. The suspension was centrifuged and the supernatant used as the source of the enzyme.

Phosphatases.—(a) *Kidney alkaline phosphatase*—Rat kidney (1 gm.) was ground with sand and an equal volume of distilled water in the cold. The suspension was then diluted to 100 ml. and centrifuged. The supernatant was used in the tests. (b) *Kidney acid phosphatase*—The same preparation which was used as the source of alkaline phosphatase was also used as the source of the acid phosphatase.

Hexokinase.—Rat brain homogenate was used as the source of the enzyme. The brain of a rat was homogenized in an all glass homogenizer with 9 ml. distilled water at 0°C. for 3 minutes. The homogenate was centrifuged at 10,000 r.p.m. for 3 minutes and the clear supernatant used in the experiments.

Oxidative phosphorylation.—Fresh rabbit kidney was homogenized with 1 to 2 volumes of M/15 phosphate buffer (Sorensen's), pH 7.7 at 0°C. in an all glass homogenizer (Colowick, Welch and Cori, 1940). 1 ml. portions of the whole homogenate were used in the experiments.

Other materials.—Alloxan monohydrate (Genatosan), Sodium ATP (Light), Fructose diphosphate (Sigma) and Cozymase (Boehringer and Soehne) were used in the experiments. Sodium pyruvate was obtained by neutralizing freshly distilled pyruvic acid with caustic soda.

Succinic Oxidase System

The succinic oxidase system consists mainly of the following two components (a) succinic dehydrogenase, and (b) cytochrome oxidase. Hopkins *et al.* (1938) showed that alloxan inhibited succinic dehydrogenase by oxidizing the active sulphhydryl groups. They also made the interesting observation that, in the presence of succinate, alloxan had no effect on the enzyme. Working with the succinic oxidase system as a whole, Bhattacharya (1954) recently showed that the previous presence of excess of glucose did not have any effect on the inhibition caused by alloxan. In the experiments reported here the effect of alloxan on succinic dehydrogenase and cytochrome oxidase was studied separately. It was observed that while succinic dehydrogenase was inhibited by alloxan (Table I), cytochrome oxidase was resistant to its action (Table II). Previous presence of excess of glucose did not offer any protection against alloxan-inhibition of the succinic dehydrogenase (Table I).

TABLE I

Effect of glucose on the inhibition of succinic dehydrogenase by alloxan

Method: Enzyme activity was measured by the Thunberg methylene blue technique. Each Thunberg tube contained in the main compartment 0.3 ml. enzyme, 0.8 ml. of 0.1 *M* phosphate buffer, pH 7.4, 0.2 ml. of 5% glucose (or 0.2 ml. of distilled water) and 0.2 ml. of alloxan (or 0.2 ml. of distilled water). The hollow stopper contained 1 ml. of 1/10,000 methylene blue and 0.2 ml. of *M*/2 succinate. After evacuation the tubes were placed in a thermostat at 38°C. for a few minutes for warming and then the contents were mixed at zero time (about 15 minutes after the addition of alloxan).

(Alloxan concentration— 2×10^{-3} *M**)

Contents of tubes				Reduction time† (min.)
Enzyme + succinate	8
Enzyme + glucose + succinate	8
Enzyme + alloxan + succinate	18
Enzyme + glucose + alloxan + succinate	18

* Alloxan is extremely unstable at higher pH (above pH 6.8), its half-life at 37°C. and pH 7.4 being about 1 minute (Patterson *et al.*, 1949). In this experiment, as well as in the majority of the other experiments described here, the pH of the reaction mixture was 7.4 or higher. The concentration of alloxan given is the one which is reached immediately after the alloxan is added to the system. This concentration exists only momentarily and falls to a very low value after a few minutes.

† There was no reduction of methylene blue by the enzyme preparation without succinate both in the presence and in the absence of glucose.

TABLE II

Effect of alloxan on cytochrome oxidase

Method: Manometric. Each Warburg vessel contained 0.2 ml. enzyme, 0.5 ml. 0.2 *M* phosphate buffer, pH 7.4, 1.0 ml. 1×10^{-4} *M* cytochrome c, 0.2 ml. water and 0.1 ml. alloxan (or 0.1 ml. water). The reaction was started 15 minutes after the addition of alloxan by adding from the side bulb 0.3 ml. 0.114 *M* Na-ascorbate. The auto-oxidation of Na-ascorbate, both in the presence and in the absence of alloxan, was determined in two other flasks which contained all the materials as described above, except that the enzyme added was previously heated for 5 minutes in a boiling water bath. Temp.—38°C. Gas—air.

Alloxan concentration (<i>M</i>)				$\mu\text{l-O}_2$ uptake/10 min.*
0	55
4×10^{-4}	56
2×10^{-3}	52

* Average of three successive 10-minute periods.

Pyruvic Oxidase

The pyruvic oxidase system depends for its activity on active -SH groups (Peters and Wakelin, 1946). Alloxan is known to have a strong affinity for -SH groups. It was, therefore, likely that the enzyme would be inhibited by alloxan, which was found to be the case. Previous presence of glucose, however, offered no protection against the inhibition caused by alloxan. The results are given in Table III.

TABLE III

Effect of glucose on alloxan-inhibition of pyruvic oxidase

Method: The enzyme activity was measured by the rate of oxygen uptake in the presence of 0.005 *M* Na-fumarate and 0.01 *M* Na-pyruvate. Each Warburg vessel contained 1.5 ml. 'brain dispersion', 0.4 ml. water and 0.1 ml. alloxan (or 0.1 ml. water). Pyruvate and fumarate were added to the reaction mixture 5 minutes after the addition of alloxan. When the effect of glucose on alloxan-inhibition was studied, each vessel contained 0.1 ml. glucose (5%) instead of the same volume of water. Temp.—38°C. Gas—air.

(Alloxan concentration— 3.1×10^{-4} *M*)

Contents of vessels	$\mu\text{l. O}_2$ uptake/30 min.	
	Without glucose	With glucose
Enzyme alone (without fumarate and pyruvate) ..	88	98
Enzyme alone (without fumarate and pyruvate) + alloxan	70	81
Enzyme + fumarate + pyruvate	210	226
Enzyme + alloxan + fumarate + pyruvate ..	128	146
% inhibition	52.5	49

(The two experiments—with glucose and without glucose—were carried out with two different enzyme preparations.)

Triosephosphate Dehydrogenase

Triosephosphate dehydrogenase is also a sulphhydryl enzyme (Rapkine, 1938; Rapkine and Trpinac, 1939). Rapkine (1938) showed that the enzyme was rendered more susceptible to oxidants after the removal of cozymase by adsorption on charcoal. The inactivation of the enzyme by oxidizing agents could be reversed by subsequent contact with reducing agents (Rapkine and Trpinac, 1939). Studies on the effect of alloxan on the enzyme showed that triosephosphate dehydrogenase, which had not previously been freed from cozymase, was resistant to alloxan action (Table IV). The cozymase-free enzyme was, however, strongly inhibited by low concentrations of alloxan (Table IV). Addition of cozymase to the charcoal treated enzyme made it once more resistant to alloxan-inhibition (Table IV). Glucose could not protect the cozymase-free enzyme against inhibition caused by alloxan (Table V). Previous presence of hexose diphosphate, however, protected the enzyme against alloxan-inhibition (Table V).

TABLE IV

Effect of alloxan on triosephosphate dehydrogenase

Method: The enzyme activity was measured by the rate of the dismutation: triose-phosphate + pyruvate → phosphoglyceric acid + lactate, in a bicarbonate medium. Hexose diphosphate was used as the instantaneous source of triosephosphate (Green *et al.*, 1937). Each Warburg vessel contained 0.8 ml. muscle extract, 0.2 ml. *M*/2 NaF, 0.3 ml. *M*/6 NaHCO₃, 0.1 ml. water and 0.1 ml. alloxan (or 0.1 ml. water). The side bulb contained 0.15 ml. *M*/2 Na-pyruvate, 0.15 ml. *M*/10 hexose diphosphate, 0.1 ml. (0.8 mg.) cozymase and 0.1 ml. *M*/6 NaHCO₃. The contents of the side bulb were tipped into the main compartment immediately after the first reading and the CO₂ evolution measured manometrically. Temp.—38°C. Gas—95% N₂ and 5% CO₂ by volume.

(a) *Enzyme preparation not freed from cozymase*

Alloxan concentration (M)						$\mu\text{l. CO}_2$ evolved/15 min.
0	68
4×10^{-4}	69
2×10^{-3}	64

(b) *Enzyme preparation freed from cozymase by charcoal treatment*

Alloxan concentration (M)						$\mu\text{l. CO}_2$ evolved/15 min.
0	71
4×10^{-4}	42
2×10^{-3}	21

(In the absence of added cozymase, there was an evolution of 19 $\mu\text{l. CO}_2$ /15 min.)

(c) *Cozymase-free enzyme preparation in which cozymase was added before alloxan*

In these experiments the 0.1 ml. (0.8 mg.) cozymase was added to the main compartment instead of 0.1 ml. water before the addition of alloxan. The side bulb contained 0.1 ml. water instead of the cozymase.

Alloxan concentration (M)						$\mu\text{l. CO}_2$ evolved/15 min.
0	60
2×10^{-3}	54

TABLE V

Effects of glucose and hexose diphosphate on alloxan-inhibition of cozymase-free triosephosphate dehydrogenase

Method: The method was in general the same as under Table IV. When the effect of glucose was studied, the experimental vessels contained 0.1 ml. glucose (5%) instead of the same volume of water before the addition of alloxan. When the effect of HDP was studied 0.1 ml. HDP (M/5) was added to the main compartment instead of 0.1 ml. water before the alloxan. The side bulb contained 0.15 ml. water instead of the HDP.

(Alloxan concentration— 4×10^{-4} M)

(a) *Effect of glucose*

						$\mu\text{l. CO}_2$ evolved/15 min.
Control	62
Control + glucose	61
Control + alloxan	37
Control + glucose + alloxan	39

(b) *Effect of hexose diphosphate*

						$\mu\text{l. CO}_2$ evolved/15 min.
Control	65
Control + alloxan	58

Lactic Dehydrogenase and Malic Dehydrogenase

The lactic acid dehydrogenase was found resistant to alloxan action (Table VI). This was to be expected as the lactic dehydrogenase is not a sulphydryl enzyme and alloxan generally inactivates enzymes by interference with the active sulphydryl groups. Malic acid dehydrogenase is a sulphydryl enzyme (Barron, 1943). This enzyme was, however, found to be only mildly affected by alloxan. 5×10^{-4} M alloxan had no effect on the enzyme, while 2.5×10^{-3} M alloxan caused about 40% inhibition (Table VII). Glucose had no effect on this inhibition by alloxan (data omitted).

TABLE VI

Effect of alloxan on lactic acid dehydrogenase

Method: The enzyme activity was measured manometrically by following the oxygen uptake in the presence of methylene blue. Each Warburg vessel contained in the main compartment 0.1 ml. enzyme, 0.8 ml. 0.1 *M* phosphate buffer, pH 7.4, 0.2 ml. *M*-lactate, 0.1 ml. water and 0.1 ml. alloxan (or 0.1 ml. water). 10 mins. after the addition of alloxan, to each vessel were added 0.4 ml. flavoprotein, 0.1 ml. (0.8 mg.) cozymase and 0.3 ml. *M*-cyanide. The centre cup contained 0.2 ml. 20% KOH. 0.2 ml. methylene blue (5%) was added from the side bulb immediately after the first reading. Temp.—38°C. Gas—air.

Alloxan concentration (<i>M</i>)						μ l. O ₂ uptake/15 min.
0	48
5×10^{-4}	46
2.5×10^{-3}	44

TABLE VII

Effect of alloxan on malic acid dehydrogenase

Method: Same as for lactic acid dehydrogenase, but with sodium l-malate as substrate instead of lactate.

Alloxan concentration (<i>M</i>)						μ l. O ₂ uptake/15 min.
0	60
5×10^{-4}	56
2.5×10^{-3}	37

Diaphorase and D-amino-acid Oxidase

These were the only two flavoproteins examined. Neither diaphorase nor d-amino-acid oxidase was found to be susceptible to alloxan action. The results are given in Tables VIII and IX respectively.

TABLE VIII

Effect of alloxan on diaphorase

Method: The activity of this enzyme was studied manometrically in the same reaction mixture as that used for the determination of lactic acid dehydrogenase. In the present experiments, however, it was the diaphorase used, and not the lactic enzyme, which was made the factor limiting the rate of O₂ uptake. Each Warburg vessel contained 0.5 ml. dialyzed extract (undiluted) of acetone-dried rabbit muscle powder, 0.2 ml. *M*-lactate, 0.2 ml. diaphorase solution, 0.7 ml. 0.1 *M* phosphate buffer, pH 7.4 and 0.1 ml. alloxan (or 0.1 ml. distilled water). 10 minutes after the addition of alloxan were added to each vessel 0.1 ml. (0.8 mg.) cozymase and 0.3 ml. *M*-cyanide. 0.2 ml. methylene blue (0.5%) was tipped in from the side bulb immediately after the first reading.

Alloxan concentration (<i>M</i>)						μ l. O ₂ uptake/10 min.
0	57
4×10^{-4}	56
2×10^{-3}	54

TABLE IX

Effect of alloxan on d-amino-acid oxidase

Method: The activity of this enzyme was also determined by measuring the rate of O₂ uptake in the Warburg apparatus. Each vessel contained 1 ml. enzyme, 0.5 ml. 0.1 *M* phosphate buffer, pH 7.4 and 0.1 ml. alloxan (or 0.1 ml. water). 0.2 ml. *M*/10 dl-alanine was added at the beginning of the readings from the side bulb.

Alloxan concentration (<i>M</i>)						μ l. O ₂ uptake/45 min.
0	74
4×10^{-4}	76
2×10^{-3}	70

Catalase

Alloxan had no effect on crude rat liver catalase (Table X).

TABLE X

Effect of alloxan on rat liver catalase

Method: 5.8 ml. of the enzyme, buffered at pH 6.8 with phosphate buffer, was treated with 0.2 ml. alloxan (or 0.2 ml. water) and allowed to stand at room temperature for 10 mins. The activity of the enzyme was then measured by the method of Von Euler and Josephson (1927) as described by Sumner and Somers (1947).

Min.	K _s (without alloxan)	K _s (with 2×10^{-3} M alloxan)
0	0.036*	0.033*
3	0.033	0.032
6	0.032	0.030
9	0.030	0.029
12	0.028	0.028

* By extrapolation.

Phosphatases

Alkaline phosphatase (kidney).—This enzyme was rather strongly inhibited by low concentration of alloxan (Table XI). Burgen and Lorch (1947) also made similar observation with purified hog kidney phosphatase. Glucose, however, could not protect the enzyme against alloxan-inhibition (Table XI). Alkaline phosphatase is not a sulfhydryl enzyme. The results thus show that alloxan can inactivate enzymes by means other than that of the destruction of active sulfhydryl groups alone.

TABLE XI

Effect of glucose on alloxan-inhibition of alkaline kidney phosphatase

Method: 1 ml. of the enzyme, 8 ml. of alkaline phosphatase substrate (Shinowara *et al.*, 1942), 0.5 ml. 5% glucose (or 0.5 ml. water) and 0.5 ml. alloxan (or 0.5 ml. water) were incubated at 37°C. for 30 minutes. Immediately after the incubation period, the protein was precipitated with trichloroacetic acid and the filtrate analysed for the inorganic P content by the method of Fiske and Subba Row (1925).

(Alloxan concentration— 1×10^{-3} M)

	Inorg. P liberated in 30 min (microgram)		
Enzyme + substrate	193
Enzyme + substrate + alloxan	82
Enzyme + substrate + glucose + alloxan	75

Acid phosphatase (kidney).—The acid phosphatase was resistant to the action of alloxan (Table XII).

TABLE XII

Effect of alloxan on acid kidney phosphatase

Method: Same as under Table XI with the exception that the acid phosphatase substrate of Shinowara *et al.* (1942) was used instead of the alkaline phosphatase substrate.

(Alloxan concentration— 1×10^{-3} M)

	Inorg. P liberated in 30 min. (microgram)		
Enzyme + substrate	47
Enzyme + substrate + alloxan	51
Enzyme + substrate + glucose + alloxan	48

Hexokinase

Griffiths (1949) showed that the ordinary mammalian hexokinase (muscle) was inactivated by alloxan and reactivated by cysteine. Thus, it is probably a sulfhydryl enzyme. Rat brain hexokinase was also found to be strongly inhibited by low concentrations of alloxan. Glucose, however, could not protect the enzyme against alloxan action. The results are given in Table XIII.

TABLE XIII

Effect of glucose on the inhibition of hexokinase by alloxan

Method: Manometric. Each Warburg vessel contained 0.2 ml. homogenate, 0.1 ml. *M*/5 MgCl_2 , 0.1 ml. *M*/2 NaF , 0.3 ml. *M*/6 NaHCO_3 , 0.7 ml. water and 0.1 ml. glucose (1% or 5%). The side bulb contained 0.1 ml. *M*/6 NaHCO_3 and 0.4 ml. *M*/40 *ATP*. When the effect of alloxan was tested 0.1 ml. of water in the main compartment was replaced by 0.1 ml. of alloxan 10 minutes before the addition of the glucose, and when the effect of glucose on alloxan-inhibition was tested, the glucose was present in the system before the alloxan was added. The *ATP* was added from the side bulb immediately after the first reading and the CO_2 evolved measured manometrically. Temp.—38°C. Gas—95% N_2 and 5% CO_2 by volume.

(Alloxan concentration— 9×10^{-4} *M*)

Contents of the flask			$\mu\text{l. CO}_2$ evolved/15 min.
0.1 ml. 1% glucose (no alloxan)	127
0.1 ml. 5% glucose (no alloxan)	126
0.1 ml. 1% glucose + alloxan	28
0.1 ml. 1% glucose + alloxan*	32
0.1 ml. 5% glucose + alloxan	33
0.1 ml. 5% glucose + alloxan*	29

* Alloxan added in the presence of glucose.

Oxidative Phosphorylation

In many tissue extracts, under aerobic conditions, there is a rapid esterification of inorganic phosphate in the presence of glucose, mannose or fructose and an oxidizable substrate like pyruvate, succinate, α -ketoglutarate, etc. (Colowick, Welch and Cori, 1940; Colowick, Kalekar and Cori, 1941). In the presence of sodium fluoride, the hexoses are converted to fructose diphosphate.

Working with rabbit kidney homogenate and succinate as the oxidizable substrate, it was found that low concentrations of alloxan produced a strong inhibition of the oxidative phosphorylation of glucose, mannose and fructose when alloxan was added to the system before the addition of the hexoses. When, however, the sugars were added to the system immediately before the addition of alloxan, there was considerable protection against the inhibition caused by alloxan. Galactose, which itself was not phosphorylated by the system, did not offer any protection against the alloxan-inhibition of glucose phosphorylation. Results of representative experiments are given in Table XIV.

TABLE XIV

Effect of alloxan on oxidative phosphorylation of the hexoses

Method: 1 ml. kidney homogenate, 0.1 ml. *M*/2 NaF , 0.1 ml. *M*/2 succinate and 0.2 ml. water in the main compartment of double armed Warburg flasks. One side bulb contained 0.2 ml. 2.5% hexoses and the other 0.2 ml. alloxan. When the effect of alloxan in the absence of the hexoses was tested, the alloxan was tipped into the main compartment 2 to 3 minutes before the addition of the hexoses, and when the effect of the hexoses on alloxan-inhibition was tested, the hexoses were added to the main compartment immediately before the addition of alloxan. Temp.—38°C. Gas—oxygen. Duration of experiment—30 min.

(Alloxan concentration— 7×10^{-4} M)

Exp. No.	Additions	P esterified (mg.)
1	Fixed at once	0
	NaF+ succinate+ glucose	1.22
	NaF+ succinate+ glucose+ alloxan	0.32
	NaF+ succinate+ glucose+ alloxan*	1.00
2	Fixed at once	0
	NaF+ succinate+ mannose	1.33
	NaF+ succinate+ mannose+ alloxan	0.35
	NaF+ succinate+ mannose+ alloxan*	0.68
3	Fixed at once	0
	NaF+ succinate+ fructose	1.21
	NaF+ succinate+ fructose+ alloxan	0.32
	NaF+ succinate+ fructose+ alloxan*	0.60
4	Fixed at once	0
	NaF+ succinate+ galactose	0
	NaF+ succinate+ galactose+ glucose	1.32
	NaF+ succinate+ galactose+ glucose+ alloxan**	0.37

* Alloxan added after the addition of the hexoses.

** Alloxan added in presence of galactose but before the addition of glucose.

In Table XV are given the results of the addition of different fractions of rat brain homogenate on the inhibition of oxidative phosphorylation of glucose by alloxan. One rat brain was homogenized in an all glass homogenizer in 5 ml. of distilled water at 0° for 5 minutes. Dilute alkali was added dropwise during the homogenization so that the pH of the homogenate never came down below 7.0. The homogenate was then differentially centrifuged in the cold so as to obtain a fraction which contained both the soluble enzymes and the mitochondria and also another which contained only the soluble enzymes.

Previous experience showed that the above preparation of soluble enzymes contained hexokinase, phosphohexoisomerase and phosphohexokinase—all the three soluble enzymes which are concerned with the phosphorylation of glucose to fructose diphosphate. The results show that addition of the soluble enzymes of rat brain did not have any effect on the phosphorylating ability of the alloxan-inhibited kidney homogenate system. The addition of the fraction of rat brain homogenate containing both the soluble enzymes and the mitochondria, however, largely restored the ability of oxidative phosphorylation to the alloxan-inhibited system.

TABLE XV

Effect of the addition of different fractions of rat brain homogenate on the inhibition of oxidative phosphorylation of glucose by alloxan (7×10^{-4} M)

Method: Essentially the same as under Table XIV. Double armed Warburg flasks were used. One side arm contained 0.2 ml. alloxan and the other 0.1 ml. glucose (5%) plus 0.4 ml. rat brain extract (or 0.4 ml. distilled water). Total volume 2 ml. Temp.—38°C. Gas—oxygen. Duration of experiment—30 min.

(Alloxan concentration— 7×10^{-4} M)

Additions	P esterified (mg.)
NaF+ succinate+ glucose	1.00
NaF+ succinate+ glucose+ alloxan	0.27
NaF+ succinate+ glucose+ alloxan+ rat brain extract (mitochondria-free)	0.27
NaF+ succinate+ glucose+ alloxan+ rat brain extract (with mitochondria)	0.65

DISCUSSION

It was earlier observed (Sen and Bhattacharya, 1952; Bhattacharya, 1953, 1954) that previous or simultaneous administration of large doses of glucose, mannose or fructose protected animals from diabetes caused by alloxan. Glucose was the most effective, mannose less than glucose, whereas, fructose was much less effective. Galactose or any other sugar or sugar-derivative did not offer any such protection. The results reported here show that the mechanism which brings about the oxidative phosphorylation of glucose, mannose and fructose in kidney homogenates was strongly inhibited by low concentrations of alloxan, and that the previous presence of the hexoses in the system effectively counteracted the inhibitory effect of alloxan. Here also glucose was the most effective and galactose, which itself was not phosphorylated by the system, had no protective action.

Oxidative phosphorylation of glucose consists essentially of the following—(a) phosphorylation of adenosine diphosphate to adenosine triphosphate coupled with oxidation in the mitochondria, and (b) transfer of phosphate from adenosine triphosphate to glucose to form glucose-6-phosphate catalyzed by hexokinase. Whole kidney homogenates, used in the experiments described above, contain besides hexokinase two other soluble enzymes, phosphohexoisomerase and phosphohexokinase, so that glucose is ultimately phosphorylated to fructose diphosphate.

Thus, an impairment of oxidative phosphorylation of glucose may arise from an inhibition of (1) the enzymes concerned with the oxidation of the substrate, (2) the mechanism whereby inorganic phosphate is transferred to ADP to form ATP coincident with oxidation, and (3) the soluble enzymes of the phosphate acceptor system. Alloxan does not inhibit the succinic enzyme in the presence of succinate (Hopkins *et al.*, 1938). In the experiments reported here, excess succinate was always present in the system before the addition of alloxan, and there was no diminution of oxygen consumption as a result of alloxan treatment (data omitted). Thus, the inhibition of oxidative phosphorylation by alloxan was not due to any impairment of oxidation. The results in Table XV show that the addition of a fraction of rat brain homogenate containing both the soluble enzymes and the mitochondria largely restored the ability of oxidative phosphorylation to the alloxan-inhibited kidney homogenate system. The addition of the soluble enzymes alone, however, had no such effect. These results strongly suggest that the inhibition of oxidative phosphorylation of the hexoses by alloxan was essentially due to an inhibition of the mechanism in the mitochondria which brings about the phosphorylation of ADP to ATP coupled with oxidation. Apparently, the previous presence of glucose, mannose or fructose, but not of galactose, protects the mechanism from the inhibition caused by alloxan. The mode of this protection is not, however, clear from the present data.

The results given in this paper show that, in addition to the mechanism of oxidative phosphorylation, a number of other enzymes concerned with the utilization of glucose are also inhibited by low concentrations of alloxan. Glucose, however, does not protect any of these enzymes against the inhibition caused by alloxan. These enzymes, therefore, seem not to be primarily involved in the causation of diabetes by alloxan. The system of oxidative phosphorylation is the only enzymatic mechanism in which the antagonism between the hexoses and alloxan with respect to the development of diabetes in animals is closely simulated. It is, therefore, suggested that the inhibition of the mechanism of oxidative phosphorylation in the beta cells is the ultimate cause of the diabetogenic action of alloxan. Presence of excess of glucose, mannose or fructose protects the mechanism against alloxan inhibition and thus saves the animal from diabetes.

The mechanism of oxidative phosphorylation is essential for the life of the cells; for, it is by the means of this device that the energy of oxidation is made available to the cells in the form of high energy phosphate bonds of adenosine

triphosphate, etc. Acute impairment of the process is, therefore, likely to cause profound derangement of the entire life activities of the cells, leading to their ultimate death. Also, such interferences are likely to be highly irreversible, because the very source of energy is tampered with, and as Potter (1945) points it out, each cell seems to be obligated to generate its own storage of energy. All these possibly explain the extreme acuteness and irreversibility of the diabetogenic action of alloxan.

As regards the selectivity of alloxan for the beta cells, the present results lend further support to the earlier suggestion (Bhattacharya, 1953) that it possibly arises as a result of a specifically low concentration of glucose in and around the beta cells due to the storage of insulin in these cells.

SUMMARY

1. The effects of alloxan on a number of enzymes concerned with the utilization of glucose have been studied.
2. Alloxan has no effect on the activities of the following enzymes—cytochrome oxidase, lactic dehydrogenase, diaphorase, d-amino-acid oxidase, catalase and acid phosphatase.
3. The activities of the following enzymes are more or less strongly inhibited by low concentrations of alloxan—succinic dehydrogenase, pyruvic oxidase, triosephosphate dehydrogenase (cozymase-free), malic dehydrogenase, alkaline phosphatase and hexokinase. Glucose protects none of these enzymes from the inhibition caused by alloxan.
4. The mechanism which brings about the oxidative phosphorylation of glucose, mannose or fructose is strongly inhibited by low concentrations of alloxan. Previous presence of the hexoses in the system, however, effectively counteracts the inhibition caused by alloxan. Galactose, which itself is not phosphorylated by the system, cannot counteract the inhibition of glucose phosphorylation by alloxan. The mechanism in the mitochondria which brings about the phosphorylation of ADP to ATP coupled with oxidation seems to be the site of the action of alloxan. The nature of the protection by hexoses remains unexplained.
5. It is suggested that inhibition of the mechanism of oxidative phosphorylation in the beta cells is the primary cause of the diabetogenic action of alloxan.

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THERMAL EXPERIMENTS WITH THE VANADIUM-BEARING TITANIFEROUS MAGNETITES OF MAYURBHANJ—A STUDY OF THE DIFFERENT TYPES OF CRYSTALLOGRAPHIC INTERGROWTHS

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INTRODUCTION

The vanadium-bearing titaniferous magnetite ores occurring in the Gabbro-Anorthosite suite of Mayurbhanj, India, are conspicuous for the various types of textures they exhibit, and these interesting features have been described by the author in two of his earlier publications (1954 and 1955). Of these interesting textural relationships, in which about a dozen minerals take part, the author has chosen four typical exsolution intergrowths in which different pairs of minerals are involved, viz. Magnetite-Ilmenite, Magnetite-Ulvöspinel, Ilmenite-Hematite and Ilmenite-Ulvöspinel, and has tried to find out the temperature and mode of formation of these intergrowths.

PREVIOUS WORK

Some work has already been done in this line where the workers have studied such crystallographic intergrowths by thermal experiments. Ramdohr (1926) was the earliest among them and with the Taberg titaniferous magnetites he found out that crystallographic intergrowths between magnetite-ilmenite, magnetite-spinel and ilmenite-hematite have originated by unmixing during the cooling down of the magma. The temperature of formation of the magnetite-ilmenite and magnetite-spinel was found to be 700° to 800° and 800° or slightly above respectively. The ilmenite-hematite intergrowths placed another problem because the hematite lamellae within ilmenite belonged to two generations. The coarser were considered to be of the first generation and the finer to be of the second by Ramdohr. According to Ramdohr the first generation of hematites forms at about 700° due to the lower solubility at that temperature while the lamellae of the second generation are due to a change in symmetry of the minerals on further cooling to a temperature between 600° and 700°. Ramdohr carried out these experiments in air and in nitrogen atmosphere.

Schwartz (1927) carried out thermal experiments with oriented intergrowths of chalcopyrite and cubanite and determined the temperature of formation of the intergrowths, as well as the nature of the mixed mineral formed.

Kamiyama (1929) also investigated into the temperature of formation of the magnetite-ilmenite intergrowths with the titaniferous magnetites of Korea. His results do not tally with those of Ramdohr (1926). He found that on heating titaniferous magnetites the widmanstätten intergrowths between magnetite and ilmenite were pronounced up to 1,150° but melting began at 1,390°.

Among other workers Edwards (1938), Greig (1932), etc., investigated into the details of the origin, nature and temperature of formation of the oriented

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intergrowths of the ore minerals and all their contributions make up the present state of knowledge in this line.

THE INTERGROWTHS

A short description of different intergrowths is given below:—

1. *Magnetite-Ilmenite intergrowth:*

Widmanstätten intergrowth in which ilmenites occupy the octahedral plane of the magnetite in the form of needles, both coarse and fine, making an angle of 60° to 65° among themselves (Fig. 1). The ratio of the two minerals is, almost always, constant.

2. *Magnetite-Ulvöspinel intergrowth:*

In this intergrowth needles of ulvöspinel are oriented in the cubic (100) plane of the magnetite making an angle of 90° among themselves (Fig. 2).

3. *Ilmenite-Hematite intergrowth:*

Here many fine needles of hematite are oriented in the ilmenite parallel to the base (0001) (Fig. 3). The hematite needles have parallel and simultaneous extinction position with that of the ilmenite.

4. *Ilmenite-Ulvöspinel intergrowth:*

Ulvöspinel needles are oriented in the ilmenite in the same way as the hematite in the latter (Fig. 4).

Heating experiments with the intergrowths:

The different intergrowths mentioned above present a problem before the investigator—the problem to determine the temperature of formation of these intergrowths as well as the paragenesis of the ore minerals. The determined temperatures of formation are, however, not precise values, because they have been measured from a change of phase at atmospheric pressure and the possible effect of pressure has been neglected. But the flexibility range of 50°C . allowed with each temperature of formation determined by the author, is probably enough for any such possible difference. The author therefore attempted thermal experiments to solve this problem and the results are compiled in the lines to follow.

The polished specimens were at first studied, the intergrowths spotted and photographed, and then subjected to heat treatment in a platinum wound tubular horizontal type of electric furnace. The temperature in the furnace is highest in the central portions, while the temperature gradient is on the decline towards the extremities. A Wheelco Capacilog controller was there for controlling the temperature and to make it constant at any fixed point within $\pm 1^\circ\text{C}$. The temperature was recorded by Pt-Pt 10% Rh Thermocouple. The heating was carried out in vacuum. The specimens were heated for 24 hours at fixed temperatures which varied from 500°C . to 1200°C . at a difference of 50°C . each time. After heating, the specimens were taken out from the furnace and chilled immediately in water, to keep the texture etc. at that temperature intact. The specimens were then repolished and studied under the microscope in the usual way. The results are stated as follows:

Temp. 500°C . Time 24 hours. In vacuum.

No noticeable change. All the intergrowths kept intact.

Temp. 550°C. *Time* 24 hours. *In vacuum.*

No appreciable change. Only the fine hematite lamellae within ilmenite are very slightly enlarged. Other intergrowths are intact.

Temp. 600°C. *Time* 24 hours. *In vacuum.*

The fine hematite lamellae are definitely affected. Shows signs of homogenization with ilmenite. Other intergrowths unaffected.

Temp. 650°C. *Time* 24 hours. *In vacuum.*

Hematites in ilmenite vanished. The two have homogenized into a new mineral which is brown in colour and anisotropic. No change in reflectance from that of the ilmenite observed. This mineral may be titano-hematite (Ramdohr, 1926). Other intergrowths are not appreciably affected.

Temp. 700°C. *Time* 24 hours. *In vacuum.*

The ilmenite lamellae in magnetite grew wider (Fig. 5). No appreciable change in colour of the fine ilmenite lamellae or the magnetite. But the colour of the big ilmenite grains change to brownish pink. The ulvöspinel lamellae in both ilmenite and magnetite unchanged.

Temp. 750°C. *Time* 24 hours. *In vacuum.*

Almost same as in 700°C. Only the ilmenite lamellae in magnetite became more dilated and show signs of disintegration (Fig. 6).

Temp. 800°C. *Time* 24 hours. *In vacuum.*

The ilmenite lamellae in magnetite definitely disturbed and homogenization has started (Fig. 7). The ulvöspinel lamellae in both magnetite and ilmenite widen and the colour of the latter two minerals change to brownish pink.

Temp. 850°C. *Time* 24 hours. *In vacuum.*

The ulvöspinel lamellae show signs of disintegration and decomposition. Homogenization of ulvöspinel and magnetite and ulvöspinel and ilmenite have started.

Temp. 900°C. *Time* 24 hours. *In vacuum.*

The disturbance in the ulvöspinel lamellae in both magnetite and ilmenite is more pronounced. The ilmenite lamellae in magnetite has almost vanished. Only traces are there to show the previous nature of the widmanstätten texture (Fig. 8).

Temp. 1,000°C. *Time* 24 hours. *In vacuum.*

The finer lamellae of ilmenite are mixed up and form a spongy and felted mass (Fig. 9). Colour of ilmenite changed to brown.

Temp. 1,100°C. *Time* 24 hours. *In vacuum.*

Almost complete homogenization of all the intergrowths (Fig. 10).

Temp. 1,200°C. *Time* 24 hours. *In vacuum.*

Complete homogenization of the intergrowths. The homogenized ilmenite-magnetite has no ilmenite lath present in it (Fig. 11) and its reflectance is about 20% in green light in air in the Berek's slit microphotometer. The mineral is

probably titanomagnetite which with lowering of temperature unmixed into magnetite and ilmenite.

Efforts have been made to get back the intergrowths from these mixed minerals by annealing the specimens heated to 1,200°C. instead of quenching it at that temperature. The annealing rate had been fixed at $1\frac{1}{2}$ °C. per minute and the specimens were annealed from 1,200°C. to different temperatures ranging from 1,000°C. downwards at the interval of 50°C. when they were quenched. Magnetite-ilmenite intergrowth started to appear at 750°C. though some few specks of ilmenite were found to exsolve in the specimen annealed to 800°C. and quenched at that temperature. By measuring the size of the ilmenite lamellae in both the original unheated intergrowths as also in the annealed specimens it was decided that the rate of cooling of $1\frac{1}{2}$ °C. per minute was most suitable. The hematite-ilmenite intergrowth was also restored partially at about 600°C. after annealing the specimen similarly. No definite pattern of intergrowth, however, could be restored in the case of ulvöspinel-magnetite or ulvöspinel-ilmenite intergrowth.

From these annealing experiments it could be confirmed that the magnetite-ilmenite intergrowth had formed at a temperature which ranged from 750°C. to 800°C. while the temperature of formation of the ilmenite-hematite intergrowth lay between 550°C. and 600°C.

DISCUSSION

From the above experiments it is evident that among the intergrowths the earliest to form were those between ulvöspinel and magnetite and ulvöspinel and ilmenite between 850°C. and 900°C. Magnetite-ilmenite intergrowth formed later between 750°C. and 800°C. whereas the ilmenite-hematite intergrowth crystallized even later—somewhere between 550°C. and 600°C. Accordingly among the primary minerals the big ilmenite grains and the magnetite grains formed first with the ulvöspinel lamellae exsolved in them. The ilmenite lamellae in the magnetite exsolved in a lower temperature while the hematite rods in ilmenite came out in a still lower temperature.

About the formation of the intergrowths of magnetite-ilmenite, ulvöspinel-magnetite and ulvöspinel-ilmenite, the process is very simple. In the decreasing temperature condition these intergrowths were formed by unmixing and the minerals present in minor amount were oriented in the crystallographic planes of those present in larger amount. The ratio of the members in the intergrowth are in all cases constant. In the case of ilmenite-hematite intergrowth, however, the process is debatable. The hematite lamellae, in this case, are very fine and their temperature of formation has been fixed between 550°C. and 600°C. In all the characters and mode of occurrence they correspond to the second generation lamellae of hematite in ilmenite as described before. As already stated Ramdohr (1926) suggested that these lamellae of hematite were formed by a change of symmetry within the minerals due to lowering of temperatures. Greig (1932) differed with Ramdohr as regards the origin of the second generation hematite lamellae due to change of symmetry. His arguments are that, firstly, the inversion temperature will decrease with the increase of Fe_2O_3 in the solid solution and there is no data which corresponds the lowering of inversion point to a given amount of Fe_2O_3 . Secondly the inversion in ilmenite demands an abrupt drop in the solubility of FeTiO_3 in Fe_2O_3 , which is not possible unless it corresponds to a phase change, i.e. an inversion in the Fe_2O_3 solid solution. At the temperature at which the saturated solid solution of Fe_2O_3 in FeTiO_3 is assumed to invert with unmixing, an equilibrium diagram consistent with the assumption would show the intersection of the curves representing the compositions of the Fe_2O_3 solid solutions which can exist in equilibrium with the high and low temperature forms of FeTiO_3 solid solutions. There would not be a horizontal line joining these curves as shown by Ramdohr (1926). An abrupt decrease in solubility of FeTiO_3 in Fe_2O_3 does not follow from

the assumption of this inversion in ilmenite. Such an abrupt decrease in solubility requires the additional assumption of an inversion in Fe_2O_3 the temperature of which is lowered by solid solution of FeTiO_3 . Any such abrupt change of solubility would take place at different temperature in the two solid solutions.

Though, as stated before, the hematite-ilmenite intergrowth in this case roughly corresponds with Ramdohr's second generation hematite-ilmenite intergrowth described already, both by appearance and by relatively low temperature of formation, the author does not think that Ramdohr's explanation as to their formation is, at least, the only plausible one. The hematites may only be an exsolved phase in the ilmenite due to lowering of temperature just as the ilmenites are in the magnetite. Moreover due to the tiny nature of the needles the melting was easy to detect in them at the very initial stage and hence Ramdohr's two generations of hematite may, in fact, belong to one generation only as it is difficult to detect melting in the larger grains from the very commencement.

SUMMARY

The author has subjected crystallographic intergrowths of four pairs of minerals in the titaniferous magnetite ores of Mayurbhanj to thermal experiments by heating them in vacuum at varying temperatures from 500°C. to 1,200°C. for 24 hours and has come to the following conclusions:—

- (i) The intergrowths of magnetite-ilmenite, magnetite-ulvöspinel, ilmenite-hematite and ilmenite-ulvöspinel have originated by unmixing in decreasing temperature condition.
- (ii) The intergrowth of magnetite-ulvöspinel and ilmenite-ulvöspinel formed first at a temperature lying between 850°C. and 900°C. The magnetite-ilmenite intergrowth formed after it between 750°C. and 800°C. only to be followed by ilmenite-hematite intergrowth between 550°C. and 600°C.

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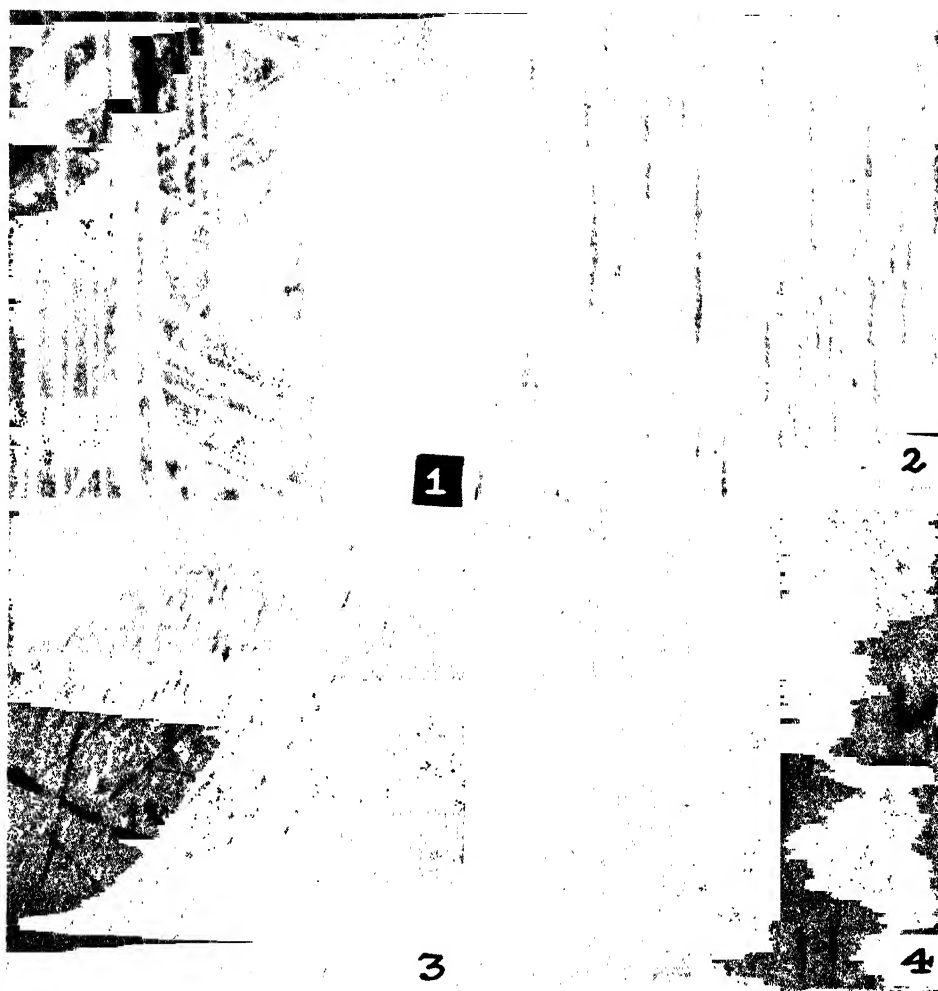
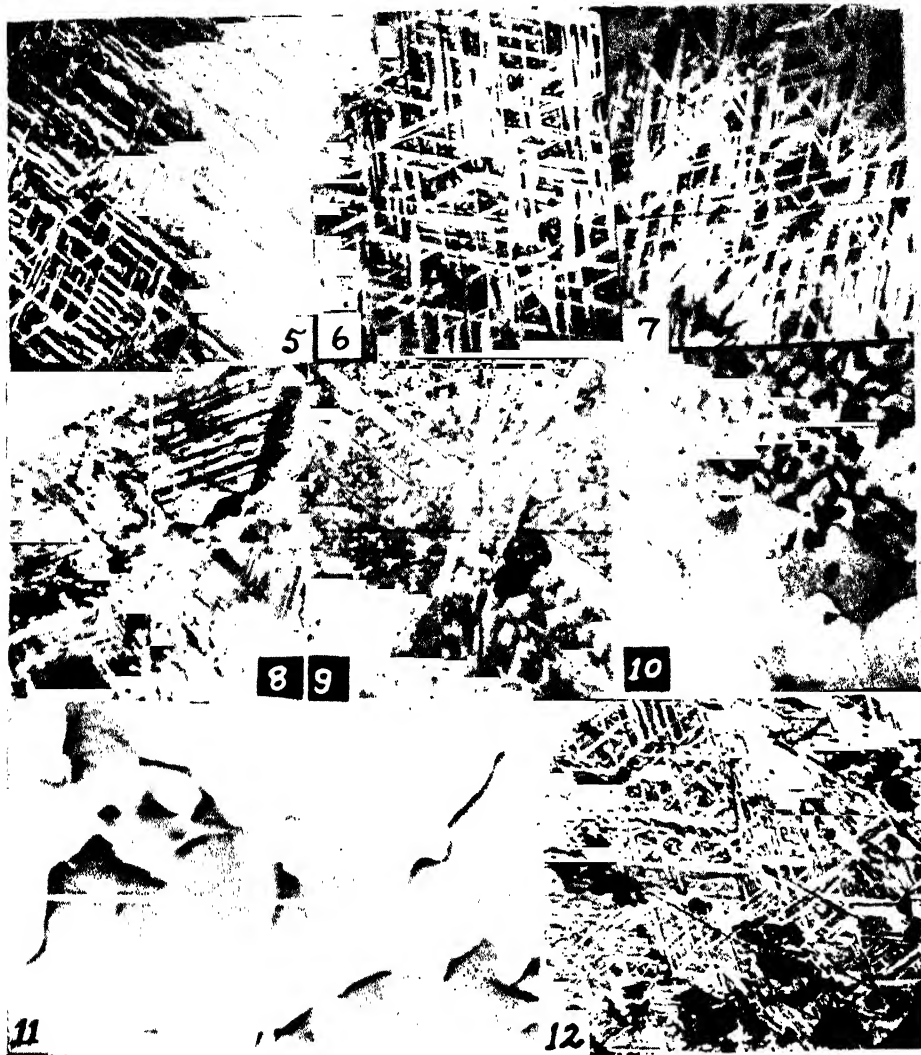


FIG. 1. Widmanstätten intergrowth of ilmenite (pale grey rod shaped lamellae) and magnetite. Oil immersion, $\times 800$.
.. 2. Crystallographic intergrowth of ulvöspinel (unetched pale grey needles) and magnetite (etched sooty black). Oil immersion, $\times 800$.
.. 3. Crystallographic intergrowth of hematite (white needles) and ilmenite (pale grey). Oil immersion, $\times 1,400$.
.. 4. Crystallographic intergrowth of ilmenite (pale grey) and ulvöspinel (dark grey needles). Oil immersion, $\times 1,400$.



- FIG. 5. At 700°C. the ilmenite lamellae (white) have grown slightly wider in the magnetite (etched black). Oil immersion. $\times 800$.
- .. 6. At 750°C. Ilmenite lamellae (white) in magnetite more diluted and show signs of disintegration. Oil immersion. $\times 800$.
- .. 7. At 800°C. Ilmenite lamellae in magnetite definitely disturbed and homogenization has started. Oil immersion. $\times 800$.
- .. 8. At 900°C. The ilmenite lamellae in magnetite almost vanished with crumbled mass at places. Oil immersion. $\times 800$.
- .. 9. At 1,000°C. The finer ilmenite mixed up in a spongy and felted mass. Oil immersion. $\times 1,200$.
- .. 10. At 1,100°C. Almost complete homogenization of the intergrowth. Oil immersion. $\times 800$.
- .. 11. At 1,200°C. Complete homogenization of ilmenite and magnetite. Oil immersion. $\times 800$.
- .. 12. The ilmenite-magnetite intergrowth revived by annealing the specimen from 1,200°C. to 600°C. at the rate of $1\frac{1}{2}$ °C. per minute. Oil immersion. $\times 800$.

EXPERIMENTAL AND ECOLOGICAL STUDIES ON THE ADAPTATION OF *MYTILUS EDULIS* L. TO SALINITY FLUCTUATIONS *

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INTRODUCTION

Most marine animals are subject to a relatively constant environment with respect to such factors as salinity, temperature, chemical composition and density. Pike and Scott (1915) pointed out this fact and stated that animals living in the ocean have little need for changes in adjustment.

Estuarine animals, however, are exposed to drastic environmental changes due to tides; and many of these animals show remarkable powers of adjustment. Perhaps the most important environmental change is salinity. For example at Conway (North Wales) the salinity ranges from 32.36‰ at spring tide to so low as 1.40‰ at neap tide (Dodgson, 1928). Estuarine animals therefore must meet the otherwise harmful effects of rapidly changing salinity upon the tissues. The present study is concerned with the manner in which the common edible mussel, *Mytilus edulis* L., reacts to such changes. Investigations were undertaken with the following main objectives:

- (i) to determine the range of salinity to which the mussel beds in river Blyth, Northumberland county, England, were naturally exposed,
- (ii) to determine the salinity tolerance range in the laboratory,

* This paper represents a portion of a thesis submitted to the University of Durham, England, in partial satisfaction of the requirements for the degree of Doctor of Philosophy.

- (iii) to study valve opening mechanism in relation to external conditions and the nature of stimulus (or stimuli) which control this; and
- (iv) to determine the efficiency of the valve closing mechanism in isolating the animal from its environment.

2. HABITAT AND SALINITY RANGE IN RIVER BLYTH

The river Blyth, Northumberland, England, is a narrow stream communicating with the North Sea by a single opening and the harbour of Blyth is situated at the mouth of the river. At low tide the depth of the river, within the range of mussel beds, does not exceed four to five feet. The average width in that area is about 120 feet.

As Lebour (1907) states, the nearly enclosed narrow harbour, with numerous piers and woodwork, affords much protection to mussels, and pebbles and sandy mud in the area offer excellent conditions for them to thrive. The mussel beds extend westwards from New Coaling Staithes, located near the mouth, to Cowpen Pool, a distance of about nine furlongs. Besides *Mytilus*, a number of other molluscs are also found in the mussel beds, the commonest among them being *Cardium edule*, *Littorina littorea* and *L. rudis*.

The beds are exposed within each tidal cycle and collections were therefore made at low tides. In all six visits were made to the Blyth estuary. At the time of each collection, samples of mantle water (retained in the mantle cavity) from two to three mussels were taken as well as the samples of water from the river stream nearest to the respective mussel beds for salinity determinations.

The salinity values for two ends of the beds are shown in Fig. 1. The lowest value (in August 1946) was obtained immediately after very heavy rains. It may

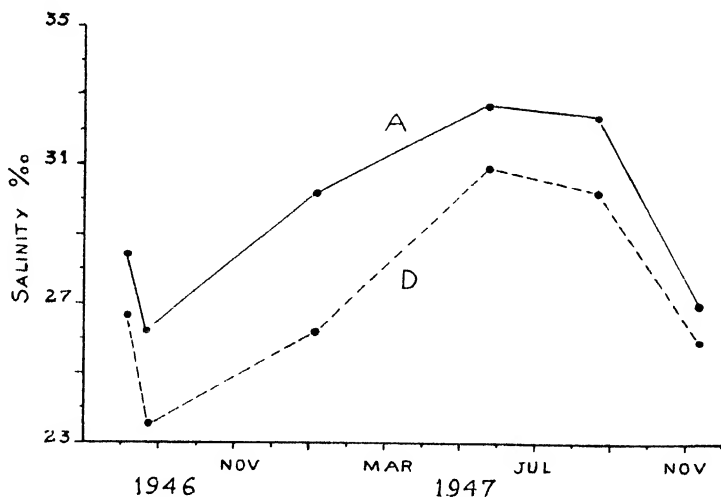


FIG. 1. The salinity at low tide within the range of mussel beds in the river Blyth. A downstream limit of beds, D upstream limit.

be seen from the figure that the salinity fluctuations, within the range of the mussel beds, were not great, the maximum difference being 3.93‰ in January 1947. Again the difference in salinity of water at low tide and at high tide was not great.

At a river area the difference never exceeded 3.52‰.* Thus the mussels do not encounter extreme changes in salinity during the course of a day's tides.

3. MATERIAL AND METHODS

The material used in the present study consisted of about 1,500 specimens of *M. edulis*, ranging in length from 4.2 to 7.5 cms. The collections were made during the period from August 1946 to January 1948 at four points along the south bank including the upper and lower limits of distribution and two other points evenly spaced between them. Mussels were washed and kept for a week in a large cement tank, supplied constantly with running seawater. The period of one week was assumed to be sufficient to acclimatise them to normal seawater.

After a week, the healthy mussels were kept in large battery jars (about 11" × 12" × 10"), containing five litres of constantly aerated seawater, for 24 hours before the experiments were commenced. By the term 'healthy' is meant those mussels that were strongly attached by means of byssus strands. In all the experiments described in the present communication, observations on the reaction of mussels in normal seawater, to which they were previously acclimatised, under different experimental conditions were also made simultaneously for comparison.

Distilled water was used for dilutions of normal seawater and whole sea salt was added to seawater for obtaining higher concentrations in all the experiments described. No extra food was supplied during any of the experiments, though seawater used was always fresh and unfiltered; but much algal growth, which might have provided some food, was established on the sides of the jars specially when the seawater was diluted. Seawater was therefore changed and the jars cleaned every 48 hours. During experiments daily records of temperature were maintained and samples of water were also collected regularly for chlorinity determination by a semi-micro method based on the Volhard Titration as described by Vogel (1948).

The empirical relationship between salinity and chlorinity, as established by the International Commission, was used for salinity calculations. In all titrations made 3 c.c. of pure acetone and 3 c.c. of conc. nitric acid were added. Replicate titrations, using samples of standard sodium chloride solution, gave standard deviation of 0.3 per cent.

4. EXPERIMENTS

I. Survival period of adult mussels in seawater of various concentrations

A number of investigators, for example Gowanloch and Hayes (1926) on *Littorina* spp., and Fox (1936) on *Mytilus californianus*, have studied the survival period of various molluscs, but none seems to have attempted to study this aspect on *Mytilus edulis* except Dodgson (1928) who made a number of interesting observations on its behaviour in waters of different salinities.

The experiments reported here were done in candy-jars of two-litre capacity containing one and one-half litres of water. Water from the mantle cavity of a mussel was drained off before keeping it in an experimental jar. Water was changed every 14 days and check on salinity was made by titration. The salinity of water in the experimental jars ranged from 0 (distilled water) to 62.43‰. Although the mouth of the jar was narrow, some amount of evaporation took place, but the

* The salinity of mantle water of mussels collected at low tide was found to compare favourably with that of river water at high tide. Accordingly salinity of mantle water has been considered for that of river water at high tide.

volume of water was maintained by addition of distilled water. The experiments were divided into three series:

- (i) two mussels per jar without aeration,
- (ii) two per jar with aeration, and
- (iii) four per jar with aeration.

In this way it was hoped to test the effects of both aeration and crowding upon the survival period.

The shell valves of a dying or dead mussel (within the size range used in present studies) are always open since the adductor muscles are relaxed and the elasticity of the ligament forces the valves open. An experimental mussel was considered dead if it did not respond to a gentle prodding with a long glass rod. Such a one was removed from the jar immediately and was substituted by a fresh marked one, of which survival period from the time of its introduction was also recorded. In all thirteen different concentrations of water were used in the experiments and the entire series repeated at least twice.

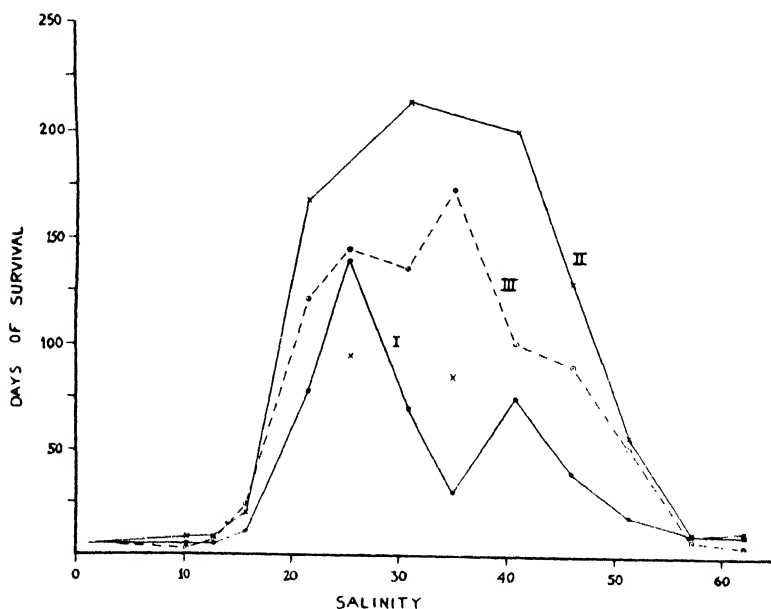


FIG. 2. The maximum survival period of mussels in various concentrations of seawater.

Curve I. 2 mussels per jar with no aeration,
 Curve II. 2 mussels per jar with aeration, and
 Curve III. 4 mussels per jar with aeration.

The results of the three series are shown graphically in Fig. 2, where each point represents the maximum survival period of the mussel. There was considerable variation in the survival period of individual mussel in the same salinity and as such it was not considered feasible to plot means. It may be seen from the figure that:

- (i) The salinity tolerance range falls approximately within the limits of 20 and 50‰. Outside these limits there is a sharp fall in resistance both to dilute and concentrated seawater.
- (ii) When aerated the effect of increase in number of mussels per jar from two to four is to decrease the survival period (cf. curves II and III).

- (iii) With the same number of mussels per jar the effect of omitting aeration is also to decrease the survival period (cf. curves II and I).
- (iv) There was considerable variation in individual response, which may have been due to the small number of mussels used. This is specially reflected in the two aberrant points for curve II, and the data are not sufficient to decide exact location of the peak of the three curves.

Aeration seemed to be an important factor for byssus formation. It was found that, without aeration, the salinity range for byssus formation was 21.52 to 45.9‰, whereas with aeration it was 15.7 to 51.2‰. Crowding, on the other hand, did not seem to have any perceptible effect on byssus formation.

II. *Time of opening the valves of mussels in relation to external conditions*

Dodgson (1928) found that in very dilute seawater of salinity less than 14.94‰, the mussels remained with their valves either closed or they opened after a lapse of a few hours and then they were apt to close again indefinitely. Milne (1940) states that, in the estuary of Aberdeenshire Dee, the valves of the mussels found at low water mark (salinity about 3.45‰) were closed. He found experimentally that mussels opened their valves within a few minutes after immersion in full strength, 75 and 50 per cent seawater, whereas when kept in 25 per cent seawater the mussels did not part their valves fully.

The experiments were therefore conducted to study in detail the relation of valve opening of mussels to the external conditions. A number of large battery jars were used with adequate arrangements for constant aeration and agitation of water. Experiments were divided into four sets comprising of observations on the opening of valves when the mussels were subjected to:

- (i) progressive (step by step) dilution of water,
- (ii) sudden dilution,
- (iii) progressive concentration, and
- (iv) sudden concentration.

The mussels were kept for 24 hours in the glass jar containing normal seawater before the experiments were begun. In sudden dilution experiments the mussels were removed from the normal seawater, dried externally, measured and placed in 31.3‰ seawater noting down the time of immersion individually. As soon as each showed signs of parting its valves, the time was recorded as also when the valves were fully open. Mussels were so arranged in the jar as to be distinguishable for subsequent observations. The mussels remained in 31.3‰ seawater for 24 hours, transferred and kept in normal seawater for 24 hours before subjecting them to the next dilution in the series, namely 26.2‰ seawater. Thus observations were made on the same animals transferred to increasing dilutions of seawater, namely 31.3‰, 26.2‰, 20.9‰, 17.35‰, 13.9‰, 10.38‰, 6.95‰ and 3.48‰ seawater, and then to tap water with 24 hours' treatment in normal seawater between each transfer.

The same technique was followed in the experiments in which the mussels were subjected to sudden concentrations of seawater of 38.8‰, 41.8‰, 44.8‰, 47.8‰, 50.8‰, 53.8‰ and 58.8‰ salinity.

The method adopted for the experiments on progressive dilution and concentrations of seawater differed only in that the transfer from one solution to the next in the series was direct without intermittent treatment in normal seawater.

At least 30 specimens were used in each of the four sets of experiments described above. Some deaths occurred in the dilute seawater but not in concentrated seawater. Apart from one death in 31.3‰ seawater, the mortality was nil in solutions more concentrated than 17.4‰ seawater. In more dilute solutions the mortality varied from 4 to 8 per cent. It is interesting to note that the mortality

(iii) During the early stages of dilution or concentration, the time taken to open the valves in a given salinity was approximately the same whether the change affected was progressive or sudden (Fig. 3). The two curves are closest together in each case before the critical salinity, as described in (ii) above, was reached. Within this range, therefore, there was little difference in the reaction to a given salinity whether the mussels were subjected to sudden or progressive changes. Beyond this region the curve begins to diverge, on the whole more obviously in the case of concentrated solutions of seawater. But in both the cases the time taken to open the valves became greater for those subjected to progressive changes than for those subjected to sudden transfers. The interpretation of these differences in terms of acclimatisation would be difficult, but it would appear that over the early part of the range, the time of 'opening' was determined by the external salinity alone rather than by the difference between this and that of mantle water, since the salinity gradient in the 'sudden changes' series was greater than in the 'progressive changes' series.

(iv) From the general shape of the two pairs of curves (Fig. 3) it would appear that when the mussels were subjected to the same salinity gradient but in opposite directions, the time taken to open the valves was about the same.

III. *Exchange between mantle water and environment*

When the mussels are placed in solutions of salinity differing from that of the mantle water, they eventually part their valves and it is natural to assume that after a certain length of time the mantle water would come in equilibrium with the external medium.

It is thus clear that, when mussels are transferred to dilute or concentrated seawater, some inhibitory stimulus from the environment must have reached the animal while the valves are still closed, since the time-lapse for reopening of valves varies in different solutions of seawater. The question now arises as to whether this stimulus results from a slight leakage—even when the valves are apparently closed—between the external medium and the mantle water resulting in some change in the concentration of the latter, or whether the stimulus acts directly from the environment perhaps on the external edge of the mantle.

To elucidate these points experiments were planned to:

- (a) determine the rate at which equilibrium is reached in different solutions after the valves are open,
- (b) determine whether any exchange occurs when the valves are forcibly closed,
- (c) find if any leakage occurs through the substance of the mantle when part of the shell is removed, and
- (d) determine the loss of water, if any, from the mantle when the mussel is exposed to air.

(a) *Exchange with valves open.*

Samples of mantle water from each of the ten mussels picked at random from a lot were taken as also the sample of seawater in which they were kept; it was established that the salinity in both the cases was the same.

A few mussels at a time were removed from seawater and placed individually in 31.35‰ seawater. Using each mussel for a single observation, mantle water samples (about 0.4 c.c., sufficient for 3 to 4 titrations) were extracted at regular intervals of 1, 3, 5, 7 and 10 minutes and then at 5 minute intervals from the time the valves had just parted as also from the time they were fully open. These mussels were not used again in any of the subsequent experiments. Thus for each time interval, six samples from six specimens were available, three for the interval

after 'begin to open' stage and the other three for the interval after 'fully open' stage.

Experiments were done with 31.55‰, 27.10‰, 20.98‰ and 17.34‰ seawater. In 17.34‰ seawater, however, mussels were found to be very sluggish in their response and only 15 out of 85 examined opened their valves fully. In this case, therefore, it was not possible to collect sufficient data for the 'fully open' stage. Mussels kept in more dilute than 17.34‰ seawater either did not open their valves or parted for a few minutes after some hours' immersion closing them again for an indefinite period.

As regards hypertonic solutions, the mussels attained equilibrium in a few minutes in 38.8‰ and 41.8‰ seawater; the maximum time taken by any individual was well under 10 minutes. Thus only higher concentrations, namely 45.41‰, 48.86‰ and 53.77‰ seawater, were considered. In 53.77‰ or more concentrated seawater mussels behaved in an anomalous manner, their response being irregular. They parted their valves very slightly after a certain variable period and reclosed them again almost immediately. After another variable pause, they reopened their

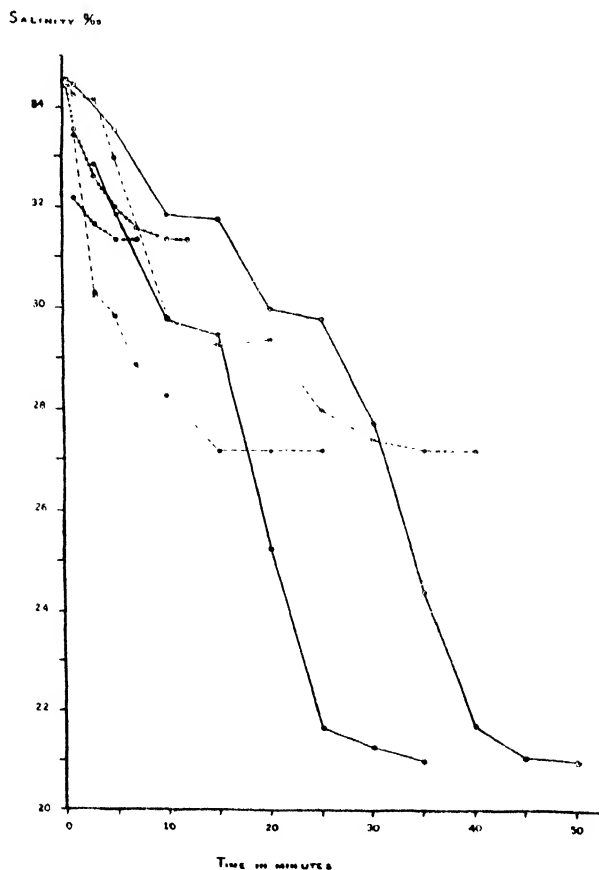


FIG. 4. The time required for the mantle water to reach equilibrium when mussels are exposed to various dilutions of seawater. Open circles 'beginning to open' stage of valves, closed circles 'fully open' stage, 'X' and line 31.35‰ seawater, dotted line 27.10‰, solid line 20.98‰.

valves rather more widely and also for a longer duration and then reclosed again. This was repeated three or four times before they finally remained open. In each successive attempt at parting, the valves were opened wider and for a longer duration. Of the 46 mussels observed, 30 reacted in the manner described above; the rest either remained closed indefinitely or opened after a lapse of a few hours.

The results of salinity determinations for various intervals are graphically shown in Figs. 4 and 5; each point in the graph represents the mean of at least three values.

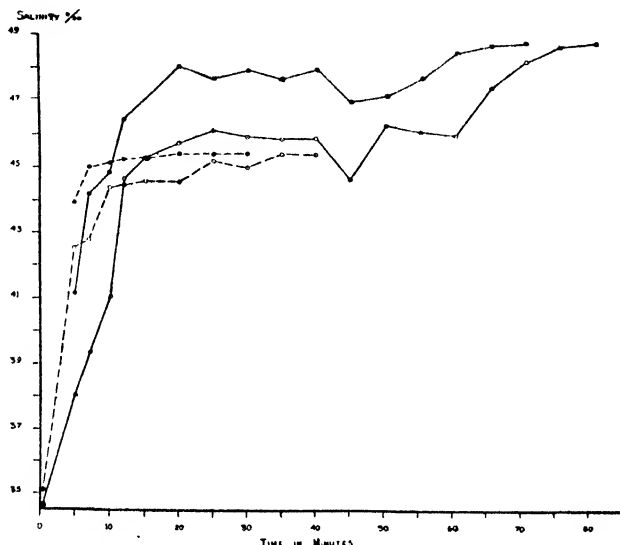


FIG. 5. The time required for the mantle water to reach equilibrium when mussels are subjected to concentrations of seawater. Open circles 'beginning to open' stage, closed circles 'fully open' stage, dotted line 45.41‰ seawater, solid line 48.86‰.

It will be seen from the two figures that equilibrium between the mantle water and the outside medium was attained in all the dilutions and concentrations tried, but the duration of time of obtaining the equilibrium varied in different concentrations of seawater. For instance in Fig. 4 it may be noted that in 31.35‰ seawater mussels reached equilibrium in ten minutes after 'begin to open' stage and in five minutes after 'fully open' stage, showing a difference of five minutes between the stages. Observations already made had shown in the case of 31.3‰ seawater that the first stage was followed in three to five minutes by the 'fully open' stage (cf. section II, figure 3), which is, therefore, not inconsistent with the five minute interval quoted above. These and the corresponding differences in the two stages in respect of other solutions tried are shown in Table 1. The figures in column 'observed time intervals between the two stages' are from the data obtained in experiments described in section II.

For all the three dilutions of seawater, the paired curves in Fig. 4 are of about the same shape, but as might be expected, they are shifted one from the other by a few minutes' interval, which is another reflection of the time lapse between the two stages. Same can be said of the paired curves in Fig. 5 for concentrations of seawater. There is the same rough correspondence between the estimated and observed time lapse for the two stages.

It is interesting to note that the equilibrium was attained in 41.8‰ and other less concentrated seawaters, in about 10 minutes. In higher concentrations

TABLE I

The time (in minutes) taken by mussels to attain equilibrium in various concentrations of water after 'beginning to open' and 'fully open' stages, showing the time lapse between these stages and comparing these with the values obtained from the experiments on the effects of salinity changes on valve opening.

Salinity ‰	Time in minutes taken for attainment of equilibrium after		Difference of time between the two stages (in minutes)	Observed time intervals between the two stages (in minutes)
	Beginning to open stage	Fully open stage		
Dilutions				
1. 31.35‰ ..	10	5	5	3-5
2. 27.10‰ ..	35	20	15	5-8
3. 20.98‰ ..	50	35	15	8-24
4. 17.65‰ ..	80
Concentrations				
1. 45.41‰ ..	35	20	15	10 (in 44.8‰ sea-water)
2. 48.86‰ ..	80	65	15	15 (in 47.8‰ sea-water)

considered this period is increased to between 20 and 80 minutes. This suggests that in water above certain salinity, the rate of the flow of the inhalent-exhalent current is retarded. There does not appear to be any evidence concerning the effect of hypertonic seawater on the beating of cilia, which might be relevant in this connection.

(b) Exchange with valves closed.

As White (1937) states the two valves of a normal mussel with periostracum intact fit very closely together so that when the shell is closed, there are no places where the two edges do not meet, and consequently seawater can be retained within the mantle (pallial) cavity. Field (1921) found that the shell, when treated with a solution of potassium hydroxide to remove all the periostracum, showed two places where the edges were not in contact. One is the byssus cleft and the other is the ligament cleft.

During some experiments it was found that when the mussels were subjected to very dilute seawater, they did not part their valves as far as could be seen by constant observations. With a view to find whether any exchange of water took place while the valves remained apparently closed, samples of mantle water from these mussels were taken for the first three hours. The results are given in Table II.

It is seen from this table that, in a number of individuals, there was very little dilution of the mantle water at least in the first hour but at the end of three hours the change was marked in almost all the cases. Apart from a few aberrant specimens, it is clear that the closed valves constitute a very adequate insulating mechanism against diffusion even when the salinity gradient is much greater than would ever be experienced under natural conditions (e.g. 34.20‰ inside the mantle cavity and 4.78‰ outside medium).

It would be interesting to know whether the small amount of leakage observed, is due to the structure of the shells or whether the valves are not in fact as tightly closed as it would appear. For this reason experiments similar to above were conducted with this difference that the valves were kept tightly closed by means of

TABLE II

Diffusion between mantle water and environment while the valves of mussels remained naturally closed.

Salinity (‰) of		Serial number of mussels	Change in salinity of mantle water (‰) of a mussel due to diffusion after	
Outside medium	Mantle water		One hour's immersion	Three hours' immersion
34.04	6.91	<i>Date: 25 June, 1947</i>		
		1	0.36	1.30
		2	0.00	1.84
		3	0.00	0.94
		4	0.36	2.02
		5	9.03	11.75
		6	0.00	1.30
		7	0.53	1.66
		8	0.00	0.94
		9	0.00	0.53
34.20	4.78	<i>Date: 27 June, 1947</i>		
		1	0.20	0.60
		2	0.60	1.42
		3	0.60	1.38
		4	0.30	3.20
		5	0.00	1.42
		6	0.38	1.42
		7	0.20	1.42
34.06	16.19	<i>Date: 1 September, 1947</i>		
		1	0.00	1.53
		2	0.39	9.19
		3	0.39	2.30
		4	0.00	0.85
		5	0.20	2.04
		6	3.06	5.56
34.09	14.26	<i>Date: 3 September, 1947</i>		
		1	2.07	3.82
		2	0.00	0.00
		3	0.00	1.88
		4	0.53	0.88
		5	1.89	2.07
		6	2.07	13.82
34.39	10.70	<i>Date: 5 September, 1947</i>		
		1	0.33	2.12
		2	0.00	0.90
		3	0.33	1.67
		4	0.19	1.28
		5	0.19	1.09

iron clamps, taking care to see that the mantle edge was not pinched between the shell valves. Seawater of 17.22‰ salinity was used as outside medium in these experiments. Samples of mantle water from 3 to 4 mussels were taken for each interval from five minutes to 96 hours after immersion in 17.22‰ seawater.

The mantle cavity of the mussels, forcibly closed, is virtually in complete isolation from the environment for at least the first 12 hours; only after 18 hours

was there some evidence of a small amount of diffusion, and even at the end of 96 hours the change, presumably due to diffusion, in the salinity of mantle water was small. The original salinity of mantle water was 34.86‰, after 12 hours it was 34.61‰, after 18 hours 34.58‰ and at the end of 96 hours 33.93‰.

It therefore follows that, under natural conditions, the valves are not and cannot be closed tightly enough to ensure complete insulation which their structure will permit, but that the closure is in fact quite adequate to provide against adverse conditions likely to be encountered in natural environment.

As regards the nature of the stimulus inhibiting valve opening in dilute seawater, the experiments summarized in Table II seem to prove that the stimulus has no connection with leakage between mantle cavity and environment. After one hour no leakage had occurred in many cases and only after three hours was there a detectable leakage in most cases. Since all the mussels remained closed during all this period, it naturally follows that the inhibitory stimulus is quite independent of a change in the concentration of the mantle water.

(c) *Exchange across the substance of the mantle with part of the shell removed.*

A number of mussels were taken and a portion of shell on one side (in the bulging area) of each mussel was removed to expose approximately 3.6 sq. cm. of the mantle, taking care not to injure the animals.

They were then tightly clamped and kept in concentrated (48.2‰) seawater. A sample of seawater was taken in which these mussels had been left overnight before transfer. Samples of mantle water from three separate specimens were collected at one hour intervals for six hours after immersion. The experiments were repeated in dilute (18.86‰) seawater. In both the experiments, therefore, the mussels were subjected to the same salinity gradient (about 15‰ difference between the mantle water and environment) but in opposite directions.

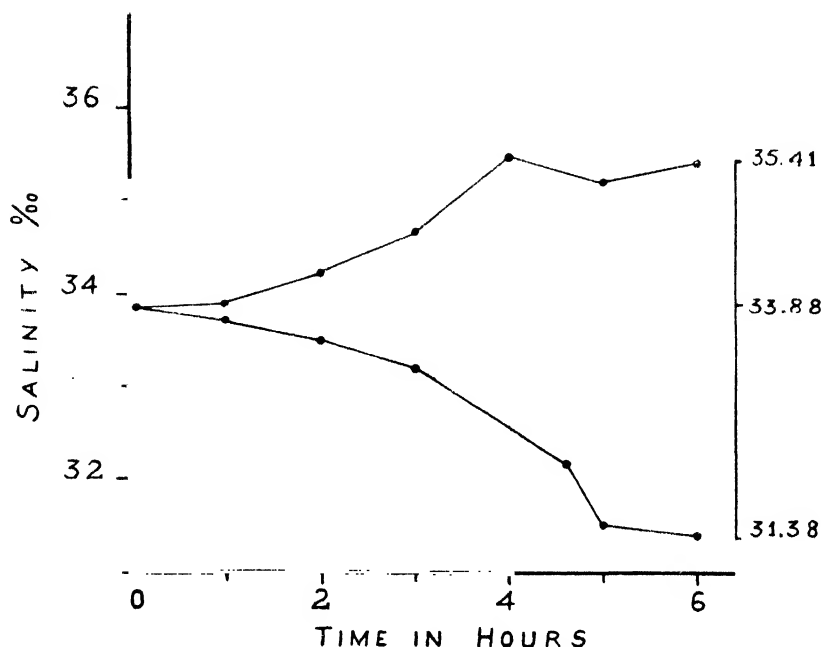


FIG. 6. Changes in the salinity of the mantle water of tightly closed mussels of which a portion of the mantle was exposed to 18.86‰ or to 48.20‰ seawater.

The results are shown graphically in Fig. 6 where each point represents the mean of three values. It can be seen that: (i) In concentrated seawater the change in the mantle water was very slight during the first two hours. At the end of six hours it was 1.53‰, giving a mean diffusion rate of 0.255‰ per hour. (ii) In dilute seawater, the change was of about the same order. At the end of six hours, it was 2.52‰, giving a mean diffusion rate of 0.42‰ per hour. The apparently greater permeability in dilute than in concentrated seawater may perhaps be associated with the deleterious effects of dilute seawater on survival. The salinity used—18.86‰—is close to the lower limit for survival (cf. Fig. 2).

The permeability of the substance of the mantle, though measurable, is nevertheless sufficiently low to prevent dangerous alteration in the concentration of the mantle water due to the exchange through the mantle itself under natural conditions. Meigs (1914) working on *Venus mercenaria*, a clam, states that 'experiments on the mantle, by which the adductor muscles are covered during the life of the clam, show that this tissue is nearly, if not quite impermeable to sodium chloride'.

(d) *Loss of water from the mantle cavity, when the mussel is exposed to air.*

A glass tray (about 9" × 7.5" × 1.5") was filled with mud—one inch thick layer—from Blyth estuary and made soft with seawater. A similar tray was filled with dry sand. A few mussels at a time were kept in each tray and exposed to the air on a bright and windy day. Samples of mantle water from three separate specimens were taken at regular intervals for six hours.

The 'wet Blyth mud' experiment was an attempt to provide conditions which could be reasonably compared with natural conditions encountered, and the other 'dry sand' experiment represented rather drastic conditions that are not likely to be encountered in nature.

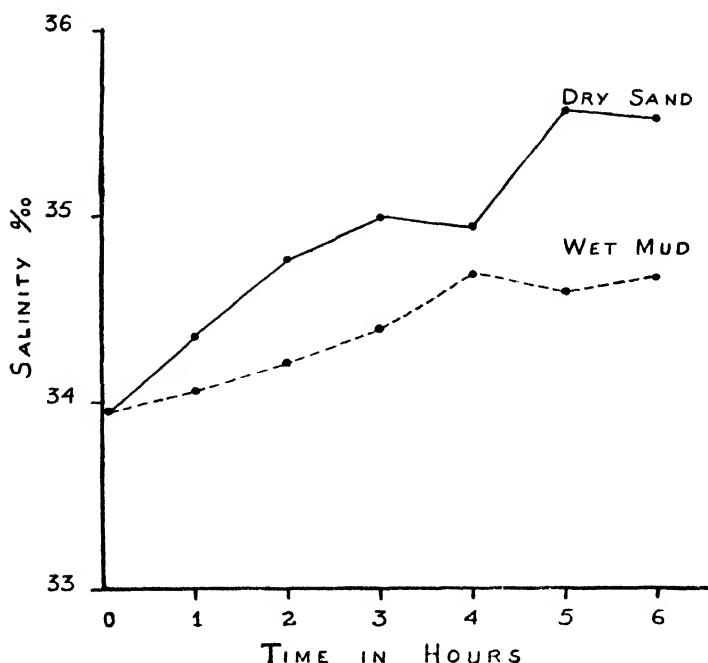


FIG. 7. Changes in the salinity of the mantle water of mussels subjected to various drying conditions.

The results are graphically represented in Fig. 7. It can be seen that:

- (i) Very little change in salinity occurred in the mantle water when mussels were exposed to the air for six hours.
- (ii) When kept exposed on 'wet Blyth mud' the change in salinity of the mantle water was from 33.98‰ to 34.67‰, a difference of 0.69‰ for six hours' exposure on a sunny and windy day, giving a mean rate of evaporation of 0.115‰ per hour as compared to 0.09‰ for the actual first hour's exposure.
- (iii) When subjected to 'dry sand' the change in salinity was 1.61‰ for six hours.

It therefore seems highly improbable that, under any conditions to which mussels might be exposed between tides, there would be any significant concentration of the mantle water due to evaporation.

IV. *Experiments to determine the nature of stimulus which opens mussels*

It has already been shown that *M. edulis* is in some way sensitive to the environmental changes even when apparently closed. The results obtained from experiments on tightly clamped mussels confirmed this. The question remains as to whether this undoubted sensitivity is primarily due to external change in the ionic concentration or due to change in osmotic pressure. To test this it was planned to substitute some of the salts by a non-electrolyte (glycerine).

A stock solution of glycerine, isotonic with full strength seawater (salinity calculated as 34.8‰), was prepared by making up 76.4 c.c. of glycerine to one litre with distilled water.

The technique followed was similar to that described in connection with experiments to determine the time of opening of valves of mussels in relation to external conditions. Mussels were subjected to isotonic solutions of 50, 40, 30, 20, 10 per cent seawater (hereafter referred to as isotonic 50% seawater, etc.) and finally pure isotonic glycerine solution. For each set of experiments fresh lots of at least 10 mussels were observed.

Summarised results of these experiments are given in Table III where deviations of average values in various dilutions of isotonic seawater solutions from the mean in normal seawater have been shown.

TABLE III

Deviations of average values in various dilutions of isotonic seawater solutions from the mean in normal seawater.

(B: beginning to open stage; F: fully opened stage)

Type of solution	Deviation of average values from the mean in normal seawater		Mean time in minutes taken by mussels to part their valves in normal seawater	
	B	F	B	F
Normal 50% seawater ..	5.93	20.60	0.75	3.68
Isotonic 50% seawater ..	0.69	3.95		
Isotonic 40% seawater ..	1.16	3.95		
Isotonic 30% seawater ..	2.28	7.57		
Isotonic 20% seawater ..	1.84	2.80		
Isotonic 10% seawater ..	3.09	4.34		
Isotonic glycerine solution ..	3.65	..		

It was observed during the experiments that:

- (i) Mussels do not always take the same length of time to part their valves when transferred from a normal seawater to another vessel also containing the same seawater. There was certain degree of variation. But there was obviously a similar trend in their response to similar conditions.
- (ii) Mussels took about the same length of time to open their valves in isotonic 50% seawater as when kept in normal seawater, which was therefore different from their response in normal 50% seawater.
- (iii) Nearly 60 per cent of the mussels did not part their valves fully in normal 50% seawater; on the other hand they did so in isotonic 50% seawater taking about the same time as when kept in normal seawater.
- (iv) Previous experiments had already showed that mussels did not part their valves in seawater more dilute than 50 per cent (i.e. 17.4% salinity), but in solutions made isotonic with full strength seawater containing 40, 30, 20 or 10 per cent seawater, the mussels opened their valves fully taking about the same time as when kept in normal seawater.
- (v) In pure isotonic glycerine solution, the mussels parted their valves but not fully. This was a very contrary reaction to that in tap or distilled water in which they remained with valves closed for indefinite period.
- (vi) Further experiments to determine the minimum of salt necessary to stimulate mussels to part their valves fully, showed that an addition of 10 per cent seawater to isotonic glycerine solution was sufficient to provide the necessary stimulus.

It would therefore seem that the osmotic pressure, and not the ionic concentration of the external medium, is the principal stimulus controlling the initial opening of valves, though for fully opening of valves, a small amount of salts, equivalent to that contained in 10 per cent seawater, is essential.

5. DISCUSSION

General considerations.—Most estuarine animals, normally subjected to more than a small change in salinity due to tide cycles, possess some regulatory mechanism in varying degree so that the harmful effects of rapidly changing salinity on the composition and concentration of the body fluid are minimised (Krogh, 1939). *Mytilus edulis* lacks osmoregulatory mechanism as is shown by the results of experiments conducted by Conklin and Krogh (1938). They state that the osmotic pressure of the blood of *Mytilus* follows closely that of diluted seawater, though blood chloride was somewhat higher than in the surrounding water. Experiments on several marine medusae have given similar results (Fredricq, 1901; Macallum, 1903; Bottazzi, 1908). Beadle (1943) states that there is, in fact, a large number of marine animals which, although lacking the mechanism for osmotic regulation essential for penetration of fresh water, can still live in much diluted seawater. *Mytilus* can withstand a considerable dilution of the blood and has been recorded from seawater of 4.50‰ salinity in the Baltic where it lives permanently (Välikangas, 1933). Experiments by Nagel (1934) on other brackish water animals, e.g. *Carcinus*, show that many species can withstand some dilution of the blood. Milne (1940) reports that, near the low tide mark, *Mytilus* was found to be living in 10 per cent seawater (3.45‰) for some time, with valves closed, thus isolating itself and its mantle water from an unfavourable environment. According to Beaudant (1816) some marine molluscs (*Patella vulgata*, *Cardium edule*, *Mytilus edulis*, etc.)

were acclimatised to fresh water by very gradual lowering of salinity taking about six months to reach perfectly fresh water, in which these animals were living healthily with fresh water forms like *Limnaea* and *Planorbis*. *Mytilus* seemed particularly resistant suggesting that during the process of acclimatisation it had perhaps developed an active osmoregulatory mechanism. These experiments, interesting as they are, must nevertheless await confirmation before drawing any definite conclusions. Even were it confirmed that the adult *Mytilus* could be acclimatised to fresh water, the fact remains that it has never established itself in completely fresh water as has been accomplished by *Dreissensia*, perhaps because, for some reason, the necessary mechanism cannot be developed in the larval stages. Some experiments on slow acclimatisation of *M. edulis* to diluted seawater were conducted, but unfortunately they could not be completed owing to an accident. However, by diluting at the rate of 5 per cent per week, the mussels were found to form byssus after three days in 30 per cent seawater (salinity 10.44‰). In 25 per cent seawater byssus formation was rather defective. It would obviously be desirable to continue these experiments and if Beaudant's claim to acclimatisation to fresh water or to a solution approaching it could be substantiated, an investigation of the osmoregulatory mechanism would be of great interest.

Variability.—In spite of the fact that the mussels were collected from the same locality, there was much individual variation among them in laboratory experiments. Bouxin (1931), working on *Mytilus edulis* and *M. (edulis) var. galloprovincialis* from the estuary of Moros (Concarneau), stresses the fact that there is a considerable individual variation in their behaviour. During the conduction of present experiments this variation in their reaction to salinity changes was quite apparent. Nevertheless the results obtained from a large number of specimens showed a definite trend in response to various experimental conditions (cf. Figs. 2 and 3), and it therefore seems justifiable to draw general conclusions.

Survival.—Fox (1936) working on *Mytilus californianus* found that the aeration is a factor very beneficial to survival of the mussel in water of any salinity that is not fatal in a short time. Crowding on the other hand, even under aeration, was found to be harmful. The results of the present experiments of the survival of *M. edulis* confirm those findings. Fox (*loc. cit.*) however postulated that crowding of individuals, found to be harmful even under conditions of continuous aeration, was perhaps due to accumulation of non-volatile waste products in the experimental jars. But the present experiments have shown that even when the water in the jars was changed every 14 days to eliminate the possible accumulation of toxic waste products, the crowding had a deleterious effect on survival.

Under conditions of continuous aeration the salinity tolerance range was found to be fairly wide, the limits being about 16‰ and 51‰ for 24 days or more. With two individuals per jar, *M. edulis* survived the maximum of 214 days in water of 30.61‰ salinity, 168 days in 21.52‰ water and 202 days in 40.52‰ water. With four individuals to a jar, the optimum salinity for maximum survival shifted from 30.61‰ water to normal seawater. *M. edulis* survived the maximum of 174 days in normal seawater (salinity 34.91‰) as against 144 days in 25.27‰.

The results on survival experiments are summarised as follows:

Lower limit for survival	.. 12.65‰ to 15.70‰.
Lower limit for normal health	.. 15.70‰ to 21.52‰ (cf. Fig. 2).
By slow dilution animals can survive in	10.44‰ water.
Lower limit for byssus formation	.. 15.70‰.
Upper limit for survival	.. 51.20‰.

These results generally support the findings of Dodgson (1928) for *M. edulis* from Conway (North Wales). The upper limit for survival, according to Dodgson (*loc. cit.*), was, however, 62.00‰. Gowanloch and Hayes (1926), working on *Littorina* spp., observed that *L. rudis* was able to live indefinitely and remain active in

15.0‰ seawater, but a salinity below 13.75‰ was fatal. In the case of the other two species, namely *L. palliata* and *L. littorea*, salinity below 12.5‰ was fatal. These values correspond roughly with the lower limits for survival of *M. edulis*. There appears to be no information available concerning the salinity tolerance range of other marine and brackish water animals which do not possess any osmoregulatory mechanism.

Nature of stimulus.—*M. edulis* has been shown to be sensitive to environmental changes even when apparently closed. The question thus arises as to the nature of the stimulus and the manner in which it reaches the animal. Three possibilities suggest themselves:

- (i) There may be some exchange of salts through a leak between the not quite water-tight edges of the mantle. The animal may, in fact, react to a slight change in the concentration of the mantle water. But the experiments seem to prove that no such change occurs, at any rate, for some time, and that, when some change eventually does occur, it has no obvious relation to opening and closing of the valves. Bouxin (1931) states that *Mytilus* spp., when subjected to unfavourable salinity changes, close their valves, and for the first two hours the consumption of oxygen for respiration is nil.
- (ii) The mantle edge may function as semi-permeable membrane and osmotic movements of water across it might cause an increase or decrease of hydrostatic pressure developed in the mantle cavity since the presence of shell-valves will not permit expansion. The animal, thus, may be sensitive to these pressure changes. An objection to this theory is that only a very minute area of the mantle edge is likely to be exposed to the surrounding medium, when the valves are naturally closed. When the area of the exposed mantle was artificially increased by removing part of the shell, the passage of water and salts across the mantle was not appreciable. In the first six hours, when subjected to a salinity gradient of about 15‰ in both the directions in relation to normal seawater, the change in the original salinity of mantle water amounted to only 1.2‰. The change of this order in the salinity of mantle water was observed in mussels kept for at least three hours in dilute seawater and during the period of observations the valves continued to remain closed (cf. Table II).
- (iii) The external edge of the mantle, which apparently is the only part of the animal likely to be exposed to the environment when the valves are naturally closed, may be directly sensitive to the changes in salinity and/or osmotic pressure of outside medium, thus making the animal 'aware' of the conditions in the environment. The results of the experiments with non-electrolyte seem to support this theory, but more evidence is required for a definite decision. No other data seem to be available which might be relevant to this aspect of the problem.

Reactions to salinity changes.—The work of Dodgson (1928) and Milne (1940) show that the animal was sensitive to dilutions of seawater and that valve opening was progressively delayed with increasing dilutions. This was confirmed in the present experiments where observations were made on a large number of specimens. The results showed that the early stages of dilutions had only a slight retarding influence on the time of opening of valves. But in waters more dilute than 17.40‰ seawater, there was considerable retardation in the time of opening, specially so in the case of mussels subjected to progressive salinity changes. This critical salinity of 17.40‰ (in relation to valve opening) roughly corresponds with the lower limit

for survival deduced from survival experiments and must be supposed to have some relation with it. In concentrated seawater solutions the critical salinity for valve opening was found to be 51.80‰, which also corresponds with the upper limit for survival.

During these experiments a few mussels died in dilute seawater and none in concentrated seawater, suggesting that fresh water has some deleterious effect on the life of the mussel in a short time. Dodgson (1928) states that the lethal effect of fresh water is somewhat remarkable, as there is evidence that mussels can keep out strong solutions of highly noxious materials, e.g. nitre cake, which was used experimentally for the purpose of cleaning them. However they may succumb if the solution of nitre cake be very weak. Their admission of fresh water may possibly be explained in the same way as that suggested in the case of very weak nitre cake solutions.

Exchange between mantle water and environment.—In very dilute or very concentrated seawater, the valves of the mussels are permanently closed. Once the valves have opened, it takes a maximum of 80 minutes to attain equilibrium in 17.65‰ and 48.86‰ seawater. These are about the two limits in which the animals will open the valves at all.

Some idea is now available as to the extent of diffusion between mantle water and external medium when the valves are closed. There is normally a slight exchange, which is quite insignificant, over a period during which the mussels are normally exposed to dilute seawater in the natural habitat of the estuary, and it can reasonably be concluded that under normal circumstances, valve closing is an adequate mechanism for isolation against a temporarily unfavourable environment.

Further, it would be interesting to know by what path such diffusion as does occur in a naturally closed mussel normally takes place. The results show that when the valves are naturally closed, the two edges of the mantle fit tightly together from which it might be supposed that the diffusion, as reflected by the change in the salinity of mantle water, is not due to a leak between it and the environmental water. The experiments involving exposure of the mantle by removal of a portion of shell, show that the mantle is in fact somewhat permeable.

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7. ABSTRACT

(i) *Mytilus edulis* L., from the Blyth estuary, are naturally subjected to a comparatively small range of salinity.

(ii) The salinity tolerance range, experimentally, was approximately 20‰ to 50‰, beyond which the mussels succumbed in 12 days or less. The maximum survival period (more than 90 days) was in waters of salinities between 20 and 40‰.

(iii) Aeration had a beneficial and crowding a deleterious effect on survival.

(iv) When transferred from normal seawater to waters of salinity ranging between 20.88 and 45.80‰, there was very little change in the mean time taken by mussels to open their valves irrespective of whether the transfer was sudden or progressive. Outside these limits the response was erratic, and the mean time of opening of valves was increased, especially so in the case of those transferred progressively. This range, shown above, was found to correspond approximately with the salinity tolerance range deduced in survival experiments.

(v) A comparison between the effects of sudden and progressive transfers would suggest that the time of opening of valves is determined by the salinity of the outside medium rather than by the difference between this and that of mantle water.

(vi) Equilibrium between mantle water and external medium was attained in all the waters of salinity between 17.5 and 48.86‰, by the inhalant-exhalant currents set up when the valves are open.

(vii) The greater the change of salinity to which the mussel was subjected, the longer the duration of time taken to reach equilibrium. The much increased time in high concentrations above 42.0‰ seawater would suggest retardation of the inhalant-exhalant currents.

(viii) With valves closed, exchange between mantle water and outside water was small, even when the salinity gradient was very steep. Valve closing, therefore, constitutes a very effective insulating mechanism to protect mussels against unfavourable salinity fluctuation likely to be encountered in nature.

(ix) With valves forcibly closed with the help of iron clamps, the exchange between mantle water and external medium was practically abolished.

(x) It was concluded that the slight diffusion, which occurred when the valves were naturally closed, was, perhaps, through the substance of the mantle and not the result of a leak.

(xi) By exposing a portion of mantle it was shown that the substance was somewhat permeable. Experiments suggested that the permeability was greater in dilute than in concentrated seawater.

(xii) When exposed to air the change in the concentration of mantle water, due to evaporation, was measurable, but it was concluded that the change was insignificant as a factor in the normal life of a mussel.

(xiii) The prolongation in the duration of time of opening of valves, when subjected to drastic changes in salinity, would indicate that the mussel is in some way sensitive to the external change, while its valves remain closed.

(xiv) The external edge of the mantle, which apparently is the only portion of the animal likely to be exposed to the outside medium when the valves are naturally closed, was, according to the results, probably directly sensitive to external salinity.

(xv) Experiments with non-electrolyte showed that the inhibitory stimulus on mussels resulted from a change of osmotic pressure rather than a change in ionic concentration in the outside medium. But a certain minimum salts were then required for subsequent full opening of valves.

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A CONTRIBUTION TO THE EMBRYOLOGY OF *VATERIA INDICA* LINN.

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INTRODUCTION

The genus *Vateria* belonging to the sub-tribe *Vateriaceae* of the family Dipterocarpaceae (Engler and Prantl, 1888) has a single species in South India, *V. indica* (Gamble, 1915). It is a large evergreen tree growing in the forests of the Western Ghats and South Canara. The stems afford a resin and the seeds an oil. The white and fragrant flowers appear from March to May and are arranged in terminal or lateral panicles. The earlier work on the family has been reviewed by Schnarf (1931) and Nagaraja Rao (1953, 1955). The present paper embodies a study of the embryology of the species in all its aspects.

MATERIAL AND METHODS

The material was collected at Theerthahalli and Narasimharajapura in Mysore State. Formalin-acetic-alcohol and Bouin's fluid were used for fixing the material and both gave satisfactory results. The customary methods of dehydration and embedding were followed. Sections were cut at a thickness of 10-16 microns and stained in Heidenhain's haematoxylin with eosin and erythrosin as counterstains. Much difficulty was experienced while cutting the material due to the growth of hairs on most parts of the flower.

HAIRS

Unicellular hairs are present on the pedicel, calyx, stamens and ovary. Those on the pedicel and the calyx lobes usually originate in the form of regular tufts, while those on the stamens and the wall of the ovary are uniformly distributed. To begin with a few of the epidermal cells usually enlarge and grow into hairs. Later the wall of the hair becomes very thick due to heavy depositions and the lumen becomes narrowed down, the nucleus occupying a basal position (Fig. 1). Stalked multicellular glands (Fig. 2) are present on the bracts and calyx lobes and their development is similar as described in *Shorea talura* and *Hopsea wightiana* (Nagaraja Rao, 1953, 1955).

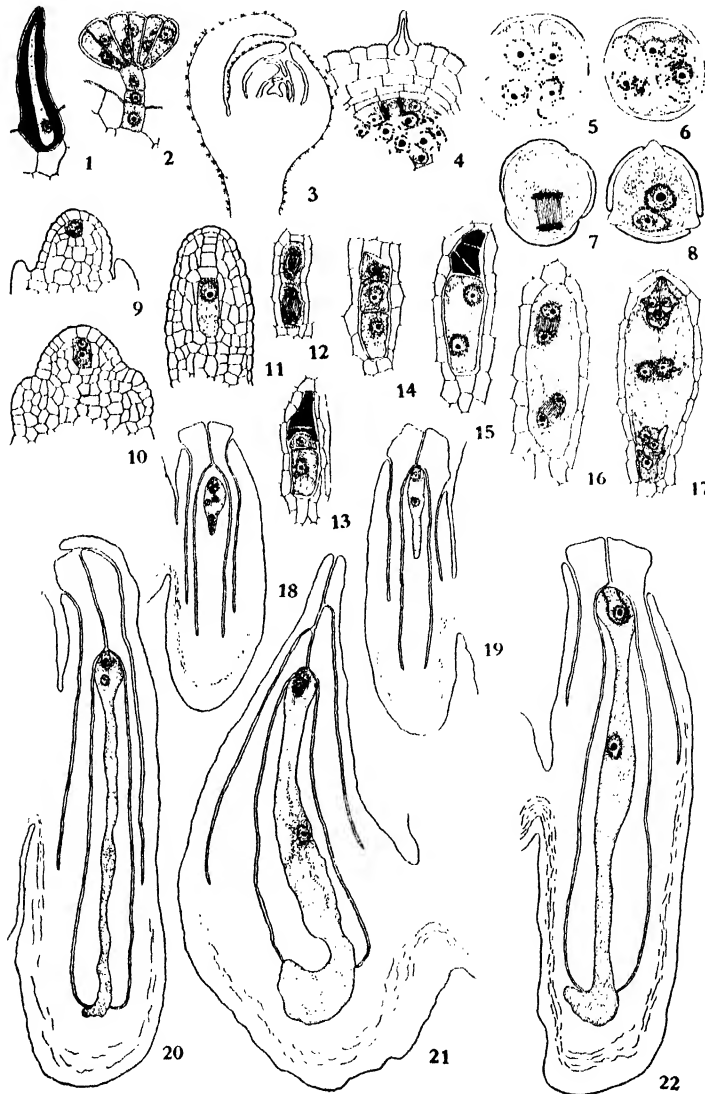
ORGANOGENY

The young floral primordium arises as a lateral dome-shaped growth in the axil of the bract. This differentiates into the floral receptacle and the pedicel. On the receptacle the calyx, corolla, stamens and carpels develop in acropetal succession (Fig. 3).

MICROSPORANGIUM AND MALE GAMETOPHYTE

The stamens are 15-30 in number. The bright yellow anther has four locules and the connective is prolonged in the form of a long hair ending in a fine point. A

transverse section of the young anther shows a plate of four hypodermal archesporial cells in each locule. They divide periclinally to form the primary parietal and primary sporogenous layers. The former undergoes repeated divisions to form the wall of the anther while similar divisions in the latter produce a large number of microspore mother cells. The wall layers thus consist of an epidermis, endothecium, three middle layers, and the tapetal cells (Fig. 4). The latter become glandular and binucleate. The cells of the epidermis as well as of the tapetum show some deposits of cutin in the form of small outgrowths. The microspore mother cells undergo the usual reduction divisions and form tetrahedral, isobilateral (Fig. 5) or decussate (Fig. 6) types of spore tetrads. Quadripartition of the mother cell takes place by centripetal furrowing.



The young microspore has a central nucleus embedded in the dense cytoplasm. As it matures the nucleus moves to the periphery and divides (Fig. 7) to produce a small lenticular generative cell and a large tube cell. The generative cell occupies the middle region of the pollen grain. The mature grain at the time of shedding is two celled, tricolpate and shows a thick exine and a thin intine (Fig. 8).

The wall of the anther is usually made up of 4-5 layers of cells. The endothecium does not develop any fibrous thickenings and dehiscence takes place by the disintegration of the wall layers as reported in *Shorea* and *Hopea* (Nagaraja Rao, 1953, 1955, 1956). The connecting cells are generally smaller and later disorganize causing the dehiscence of the anthers.

OVARY

The ovary is superior, tricarpeal, syncarpous, trilocular with one or two anatropous, bitegmic ovules in each locule. The wall of the ovary is made up of 8-10 layers of cells, the outermost of which bears a number of hairs. The ovules are attached to the central placental column and the locules are separated by incomplete septa. As observed in *Shorea talura* and *Hopea wightiana* (Nagaraja Rao, 1953, 1955) in this form also only a single ovule develops after fertilization and the rest degenerate. Degeneration of ovules may also occur before or after fertilization. Rarely all the five ovules and even the mature ones may abort. A few show a double nucellus and others have inverted micropyles, and in both the cases they are abortive. The functioning ovule develops into the seed and occupies the entire space within the fruit.

MEGASPORANGIUM AND FEMALE GAMETOPHYTE

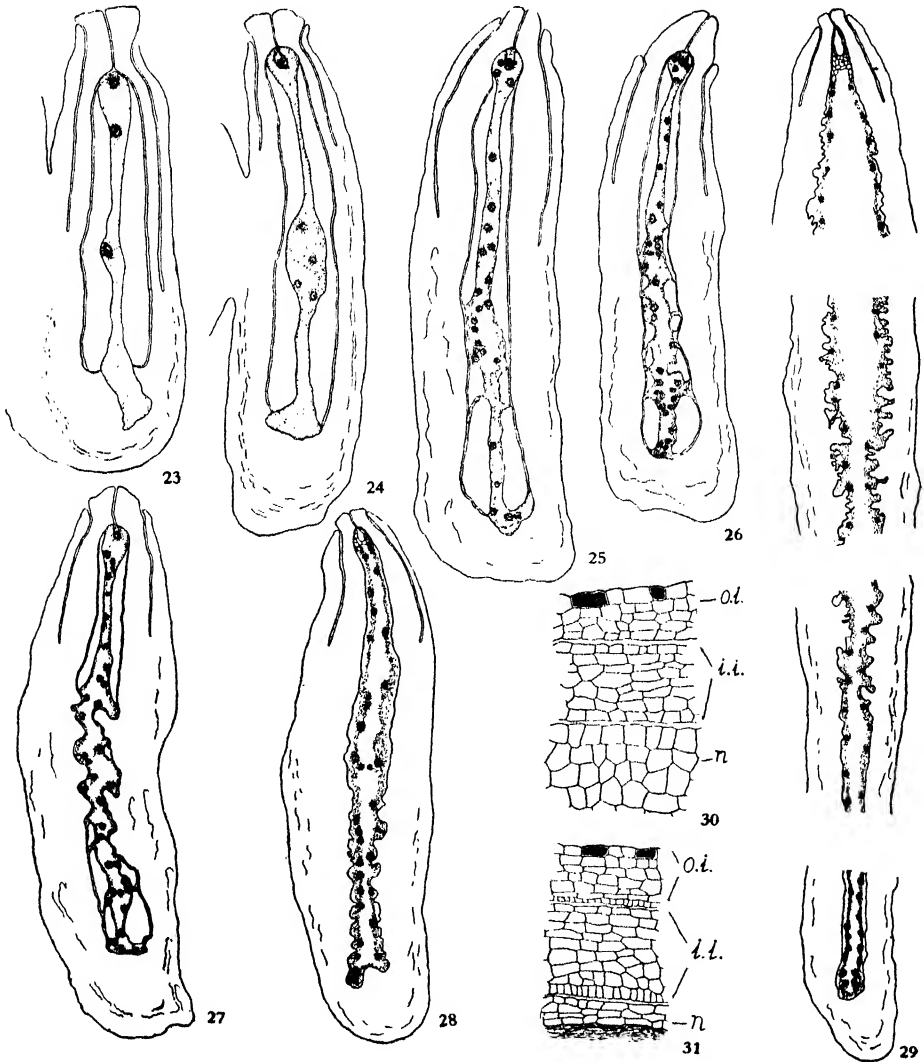
The primordium of the ovule arises on the placenta as a dome-shaped outgrowth. The inner integument develops first as a three-layered structure followed by the outer integument which consists of four layers of cells. The outer integument is arrested in its growth while the inner forms the micropyle. At the mature embryo sac stage the inner integument is seven layered whereas the outer one remains as a four-layered structure. A single vascular strand enters the base of the ovule and continues for some distance into the outer integument.

A single hypodermal archesporial cell develops (Fig. 9). This divides to produce a primary parietal cell and a megaspore mother cell (Fig. 10). Occasionally two or even three megaspore mother cells develop. The primary parietal cell gives rise to a linear row of wall cells by repeated divisions and the megaspore mother cell becomes deep seated (Fig. 11). Meiosis is normal and results in a linear tetrad of megaspores (Figs. 12, 13). T-shaped tetrads are also observed (Fig. 14). The chalazal megaspore alone functions and undergoes three divisions to form an eight-nucleate embryo sac of the Polygonum type (Figs. 15, 16) as described in other members of this family (Nagaraja Rao, 1953, 1955, 1956). The mature embryo sac (Fig. 17) is elongated. Its chalazal portion is narrow and contains the antipodal cells. The synergids are slightly hooked, and the two polar nuclei usually meet in the centre.

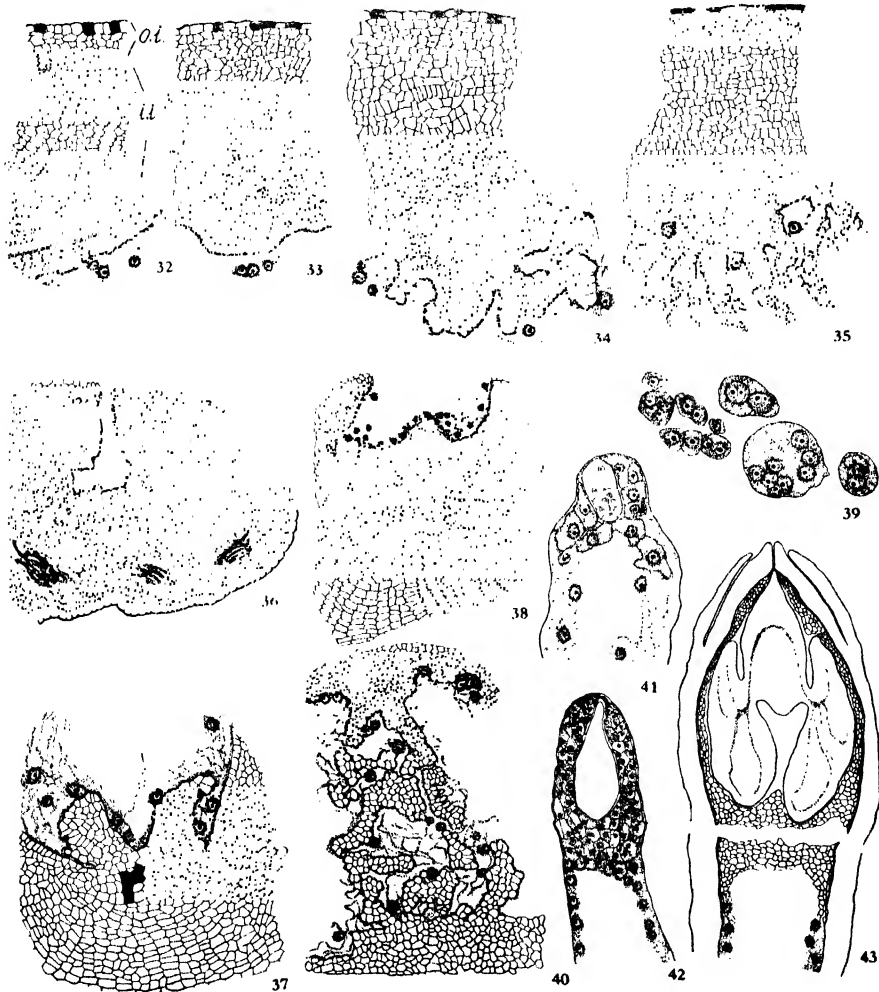
DEVELOPMENT OF SEED

At the mature embryo sac stage the ovule is an elongated structure with two integuments (Fig. 18). The embryo sac is situated in the upper region of the ovule on a massive nucellar base, whose cells have dense cytoplasmic contents. At the time of fertilization the outer and the inner integuments are 5 and 7 layered respectively (Fig. 30). During fertilization usually one of the synergids is crushed, whereas the other persists till a slightly later stage. After fertilization there is marked

meristematic activity of the cells in the basal region of the ovule and the integuments (Figs. 32, 37 and 38). As the ovule elongates the two integuments are clearly seen only in the upper region of the ovule (Figs. 23–29), whereas in the middle and lower regions of the ovule the boundaries become indistinct (Figs. 33, 34). Thus due to the all-round growth, the tissue becomes very massive, in the nucellus in the chalaza as well as the integuments. This growth also brings about the elongation of the ovule, the chalazal region being extended further and further gradually, towards the lower region of the ovule. The cells of the upper half of the integument are comparatively larger than those in the lower half. The innermost layer of the inner integument consists of cells arranged in regular series. The cells that are situated outside this layer become meristematic (Fig. 32) and are compactly arranged.



Soon after fertilization the antipodal end of the embryo sac (Fig. 18) grows to form a long tubular structure (Figs. 19, 20). Occasionally the elongation of the embryo sac may take place at an earlier stage when the egg-apparatus is still intact (Fig. 21). This outgrowth assumes a haustorial rôle and grows towards the chalaza destroying the surrounding nucellar tissue that comes in its way. When it reaches the base of the ovule it spreads (Figs. 20-24) and also destroys the basal chalazal tissue. In the meantime the middle portion of the embryo sac also expands appreciably (Fig. 24). During the active growth of the embryo sac through the nucellar tissue, the walls of these cells are destroyed resulting in their cytoplasmic contents being incorporated in the embryo sac. Oil globules and other dark stained bodies are evident in the embryo sac at this stage. The progressive and pronounced growth of the embryo sac and the subsequent disorganization of the nucellar cells, however, leaves a few scattered islands of nucellar tissue persisting in the middle and lower regions of the ovule (Figs. 25-27). Thus the nucellar tissue in the middle region of the embryo sac is the first to disorganize



followed later by the tissue in the chalazal region. The nucellus in the upper region of the ovule, however, remains unaffected for a very long time and disorganizes only at a much later stage.

When the nucellus is completely disorganized the embryo sac with the free endosperm nuclei comes in direct contact with the integument. The aggressive activity of the embryo sac does not stop at this stage but continues further along with the endosperm. It encroaches on the regularly arranged cells of the innermost layer of the inner integument, making its contour curved and wavy (Fig. 34). Further inroads of this haustorium into the integument give a highly lobed and dissected appearance to the latter. The free endosperm nuclei penetrate into these lobes. This feature was clearly made out in a number of preparations (Fig. 35).

The base of the nucellus is characterized by the possession of a definite chalazal meristematic zone of cells which becomes very active after fertilization. The growing embryo sac pierces the chalazal tissue (Fig. 36) bringing about the disorganization of the surrounding tissues (Fig. 37). The surviving portion is highly meristematic and a vascular strand traverses through it. The region becomes an extensive nutritive tissue which is gradually used up by the embryo sac after fertilization (Fig. 38).

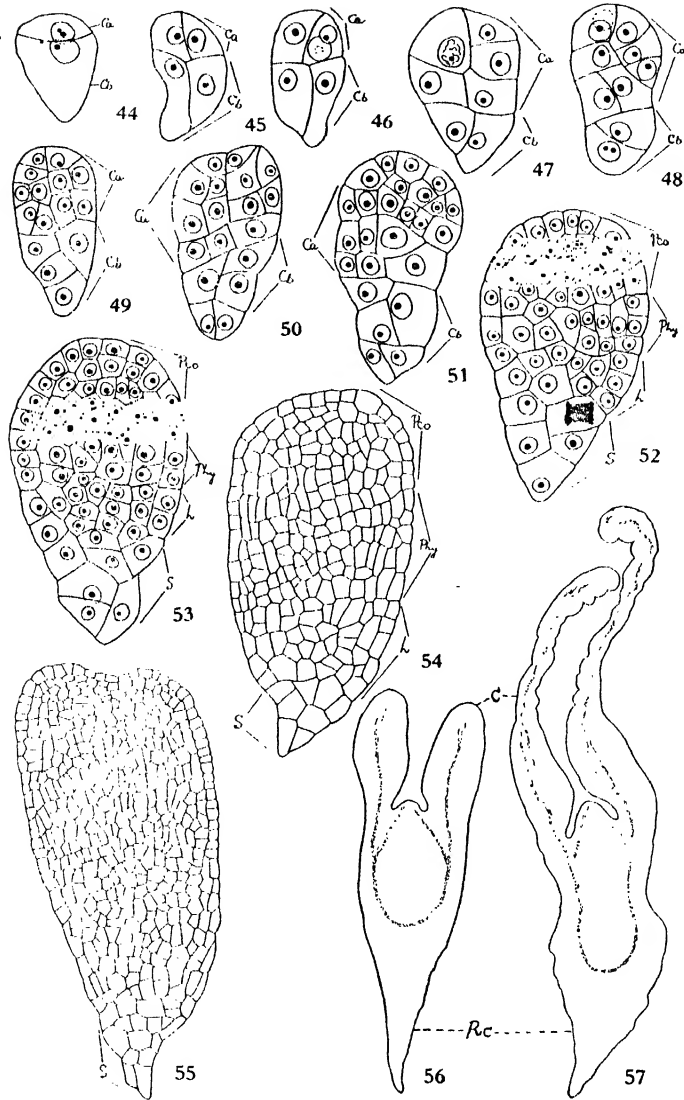
ENDOSPERM

The division of the primary endosperm nucleus takes place only after the antipodal end of the embryo sac has reached the chalazal region of the ovule (Fig. 24). The free endosperm nuclei are distributed in the peripheral portion of the embryo sac embedded in dense cytoplasm. Fig. 40 presents a surface view of the same. These nuclei are aggregated here and there in the form of endosperm cysts (Fig. 39). Each cyst consists of 2-8 endosperm nuclei. Uninucleate cysts are also found.

At an early embryonal stage, walls are laid down around the free endosperm nuclei at the micropylar end (Fig. 41). The endosperm tissue thus formed gradually extends in this region while in the basal region of the embryo sac the free nuclear endosperm persists with an aggregation of nuclei (Fig. 42). This condition is seen even in the mature seed (Fig. 43), that was dissected out from the detached fruits. At a very late stage it becomes cellular, containing plenty of oil globules.

EMBRYO

The division of the zygote is very much delayed. At this time the nucellus is mostly disorganized and the primary endosperm nucleus undergoes a large number of free nuclear divisions. The fertilized egg divides transversely to give rise to two superposed cells *C_a* and *C_b* (Fig. 44). A vertical or oblique division in each of these cells results in a group of four cells constituting a globular tetrad (Fig. 45). Further divisions in the derivatives of the terminal and basal cells are not synchronous, there being more divisions in the former. Though the first division in the terminal cell is often oblique, no epiphysis initial is separated at the quadrant stage. The embryogeny may, therefore, be considered to be irregular. Figs. 46 to 55 also indicate that the cell walls are not laid in any regular manner, most of them being oblique. However, the differentiation into the cotyledonary, hypocotyledonary, and hypophyseal regions is clearly discernible in later stages (Figs. 52, 55). The suspensor is short consisting of only a few cells. In view of the absence of any clear demarcation between the embryonal and the suspensor regions, it is difficult to decide the extent to which the basal cell contributes to the embryo proper. The early stages in the development of the embryo are similar to those in *Shorea talura* (Nagaraja Rao, 1953). Subsequently, the embryo elongates to a considerable extent (Fig. 56) and shows well differentiated, broad, unequal fleshy cotyledons inclosing the superior radicle (Fig. 57).



SEED AND FRUIT

The outer and the inner integuments of the mature seed coat are well differentiated in the upper region of the ovule, but not so in the remaining part. They consist of 5 and 7 layers of cells respectively. The cells are not compactly arranged in the integuments and these become thin and papery at a later stage. The leathery indehiscent, one seeded woody capsule has a very thick wall that consists of cells which contain plenty of tannin and resin and provides a more efficient protection to the seed than the seed coat itself.

SUMMARY

Development of the floral parts occurs in acropetal succession. Unicellular hairs are present on all parts of the flower, except the corolla lobes.

The wall of the anther consists of 6 layers of cells. The tapetal cells are binucleate and the endothecium does not show any fibrous thickenings. The pollen grains are two celled at the time of shedding.

The ovary is superior and trilocular with 2-3 anatropous bitegmic ovules in each locule arranged on an axile placenta.

The embryo sac develops according to the Polygonum type.

During the development of the seed the integuments are clearly seen only in the upper region of the ovule. After fertilization, the embryo sac grows in the form of an elongated haustorium. To begin with it destroys the nucellar tissue and, later, it also invades the integument so that its outline becomes curved and wavy.

The endosperm is free nuclear to begin with and destroys the cells of the inner layers of the integument. Later it becomes cellular in the upper part of the seed whereas in the remaining portion it persists in the free nuclear condition even in the mature seed for a very long time.

The development of the embryo is irregular. The mature embryo has two large cotyledons.

ACKNOWLEDGEMENTS

It gives me great pleasure to thank Professor P. Maheshwari for his valuable suggestions and for kindly going through the manuscript. My grateful thanks are due to Professors L. N. Rao and C. V. Krishna Iyengar, and to Dr. S. B. Kausik, for their kind encouragement and valuable guidance. I am highly obliged to my friend Sri D. A. Govindappa for having kindly collected some material for me. I am also indebted to the authorities of the University of Mysore for having awarded the University Research fellowship.

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EXPLANATION OF FIGURES

FIGS. 1-22.

- FIG. 1. Epidermal hair, mature. $\times 450$.
 FIG. 2. Multicellular gland. $\times 450$.
 FIG. 3. Stage in development of flower. $\times 50$.
 FIG. 4. Stage in development of anther wall. $\times 450$.
 FIGS. 5, 6. Isobilateral and decussate tetrads. $\times 900$.
 FIG. 7. Division of microspore nucleus. $\times 900$.
 FIG. 8. Two-celled pollen grain. $\times 900$.
 FIG. 9. L.S. young nucellus showing primary archesporial cell. $\times 180$.
 FIG. 10. Primary parietal cell and megaspore mother cell. $\times 180$.
 FIG. 11. Deep seated megaspore mother cell; note the uniseriate cells. $\times 180$.
 FIG. 12. Dyad in division. $\times 180$.
 FIGS. 13, 14. Linear and T-shaped tetrads. $\times 450$.
 FIG. 15. Two-nucleate embryo sac. $\times 450$.
 FIG. 16. Four-nucleate embryo sac. $\times 450$.
 FIG. 17. Mature embryo sac. $\times 450$.
 FIG. 18. L.S. ovule showing embryo sac. $\times 100$.
 FIG. 19. Same at a later stage. $\times 100$.
 FIG. 20. Embryo sac in contact with the basal region of ovule. $\times 100$.
 FIG. 21. Growth of embryo sac before fertilization. $\times 100$.
 FIG. 22. Enlargement of the chalazal end of the embryo sac. $\times 100$.

FIGS. 23-31.

- FIG. 23. Swollen portion of embryo sac at chalazal end of ovule. $\times 100$.
 FIG. 24. Enlargement of embryo sac in middle region of ovule. $\times 100$.
 FIGS. 25, 26. Broadening of embryo sac, note disintegration of nucellus. $\times 70$, $\times 50$.
 FIG. 27. Obliteration of nucellus in middle region of the ovule. $\times 50$.
 FIG. 28. Same at a later stage. $\times 50$.
 FIG. 29. L.S. young seed, note haustorial growths in the middle region and formation of endosperm. $\times 15$.
 FIGS. 30, 31. L.S. portion of integuments to show cellular details; note degeneration of nucellus. $\times 350$. (*o.i.*—outer integument, *i.i.*—inner integument, *n*—nucellus).

FIGS. 32-43.

- FIG. 32. L.S. portion of integuments to show cellular details; note degeneration of nucellus. $\times 350$. (*o.i.*—outer integument, *i.i.*—inner integument, *n*—nucellus).
 FIG. 33. Fused integuments and complete disappearance of nucellus. $\times 140$.
 FIG. 34. Same at a later stage showing haustorial function of embryo sac. $\times 100$.
 FIG. 35. L.S. of seed to show protrusions of embryo sac into seed coat. $\times 400$.
 FIGS. 36-38. Basal region of ovule at different stages of seed development to show growth of embryo sac, and radiating nutritive tissue. $\times 140$, $\times 315$, $\times 100$.
 FIG. 39. Endosperm cysts. $\times 500$.
 FIG. 40. Surface view of seed showing integumentary lobes and endosperm nuclei distributed in between them. $\times 40$.
 FIGS. 41, 42. Micropylar region of ovule showing formation of cellular endosperm surrounding the embryo. $\times 250$, $\times 100$.
 FIG. 43. Endosperm in mature seed. $\times 50$.

FIGS. 44-57.

- FIG. 44. Two-celled proembryo. $\times 500$.
 FIG. 45. Globular tetrad. $\times 500$.
 FIGS. 46-55. Stages in development of embryo. Figs. 46-53 $\times 500$, Fig. 54 $\times 250$, Fig. 55 $\times 200$. (*Ca*—apical cell, *Cb*—basal cell of two-celled proembryo, *h*—hypophysis, *Pco*—cotyledonary portion, *Phy*—hypocotyl region, *S*—suspensor).
 FIGS. 56, 57. Outline drawings of embryo to show differentiation of cotyledons and root cap. Figs. 56-57. 120. (*C*—cotyledons, *Rc*—root cap).

Issued June 20, 1956.

TAXONOMIC STATUS OF THE CHINESE CATFISH FAMILY CRANOGLANIDIDAE MYERS, 1931

by K. C. JAYARAM, *Junior Research Fellow, National Institute of Sciences of India, Zoological Survey of India, Indian Museum, Calcutta.*

(Communicated by S. L. Hora, F.N.I.)

(Received October 10, 1955; approved for reading on January 1, 1956)

INTRODUCTION

Peters (1880) established the genus *Cranoglanis* for a fish 280 mm. long sent to him by Dr. Gerlach from Hongkong. The chief features of this monotypic genus, with *C. sinensis* as the generic type, are the large head with a depressed snout, uncovered rugose occipital region of the head, pelvic fins with 12-14 rays each, a long anal fin with 36-41 rays, edentate palate and a free air-bladder. Peters considered his genus to be allied to the American genera *Noturus* Rafinesque and *Ameiurus* Rafinesque. Koller (1927) described from Hainan *Pseudotropichthys* as a new genus with the new species *P. multiradiatus* as its type. He did not compare his genus with *Cranoglanis*, but referred it to the subfamily Bagrina. As judged from its description and illustration, the fish is referable to *Cranoglanis*. Myers (1931) pointed out the identity of Koller's genus with that of Peters' and proposed the provisional new family Cranoglanididae to accommodate *Cranoglanis*, in view of the latter's peculiar features. Herre (1934) doubted the type-locality of *C. sinensis* being Hongkong. On the basis of material from Wuchow, Kwangsi province, China, he presumed that the original type probably came from Wuchow. However, his belief that *Cranoglanis* is not likely to occur in an island surrounded by sea-water seems questionable, specially since Koller's record is from Hainan, also an island.* In spite of the fact that the fish is said to be abundant in the West river and its tributary, the Fu river at Wuchow, China, it appears to be very poorly represented in Museums.

Myers' provision of a tentative family Cranoglanididae for this fish does not seem to have received as much attention as it deserved. Thus the *Zoological Record* for the year 1931 has overlooked this provisional family status for *Cranoglanis*, though Myers' synonymising *Pseudotropichthys* Koller with this genus has been noted. Nichols (1943) included *Cranoglanis* under Siluridae along with *Mystus* Scopoli, *Pseudobagrus* Bleeker and other genera and made no mention of a family for *Cranoglanis*. Berg (1940) in his classification merged this family with Bagridae.

TYPE SPECIES

While checking the features of *C. sinensis* for any Bagrid affinity, it was surprising to find that *Bagrus boudierius* Richardson (1845, p. 238) is also referable to *Cranoglanis*. It is noteworthy that Günther (1864) and others have erroneously

* Prof. Myers points out (*in litt.*) that both Hainan and Hongkong islands are clearly parts of a drowned continental coastline, and both possess a fauna of primary fresh-water fishes. He also states that there are probably no streams on Hongkong Island large enough to support *Cranoglanis*.

considered this species as synonymous with *Pelteobagrus vachellii* (Richardson). Richardson described *B. boudierius* from a crude drawing (Reeves 203; Hardwicke 183), a photostat copy of which is reproduced here (Pl. XVIII). The pelvic fins are seen to possess only eight rays but the drawing is known to be defective, and perhaps if the fins had been shown stretched, a few more rays would have become visible. The low set eyes, long anal fin, depressed snout and general facies clearly agree with the three specimens of *C. sinensis* examined by me.

Richardson stated that a specimen of *Bagrus boudierius* also existed amongst the collection of Chinese fishes exhibited at Hyde Park. On enquiry from the British Museum (Natural History), London, it is learnt that the above referred collection was made by Mr. Nathan Dunn and exhibited in St. George's Place, Hyde Park Corner, about 1842-5. No clue is available as to the whereabouts of the fish at present. Presumably it is lost. However, two specimens labelled as *B. boudierius* are available in the British Museum. Examination of these two specimens shows that they are wrongly identified and that they are referable to *Bagrus vachellii* Richardson. As such, Peters' fish has an earlier specific name *boudierius*.

From the above discussion, the following points are clear:

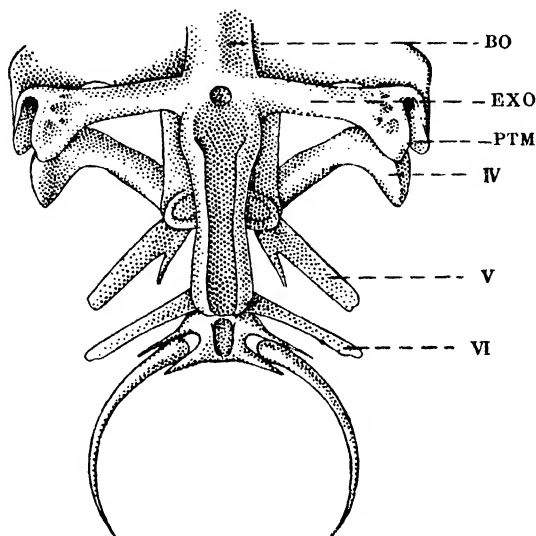
1. The generic type of *Cranoglanis* has an earlier specific name *boudierius*.
2. *Pseudotropichthys multiradiatus* Koller, from Hainan, is a synonym of *C. boudierius*.
3. The supposition that *C. boudierius* may not be found in an island is without justification.

RELATIONSHIPS

Cranoglanis possesses many external features which are common to the families Ameiuridae, Pangasiidae and Bagridae. The deeply forked caudal fin is somewhat like *Ictalurus furcatus* (Ameiuridae) but it is not so deep as in *Cranoglanis*. The high count of anal fin rays, almost straight dorsal profile, short adipose fin are as in certain Pangasiid genera. *Helicophagus* Bleeker (Pangasiidae) known from Siam, Malaya and the East Indies bears a greater resemblance to *Cranoglanis* than any other Pangasiid genera. However, the air-bladder does not possess any posterior prolongation or any multiple internal divisions as in *Helicophagus* (see Hora, 1937). The possession of four pairs of barbels, widely set nostrils, long anal fin and a free air-bladder are features common to Bagridae. *Horabagrus* Jayaram (1955) known from Peninsular India bears a greater resemblance to *Cranoglanis* than any other Bagrid genera.

The modifications of the related bones forming the lateral buttress for the air-bladder are as follows. The post-temporal bone does not extend in the form of any plate. The posterior face of the short inferior limb is slightly hollowed out. The exoccipital bones are well developed. They extend towards the inferior limb of the post-temporal bones and their distal ends are broad and somewhat flattened. The inferior limb of the post-temporal is in contact with this outer surface of the plate-like expansion of the exoccipital bones (Text-fig. 1). This plate-like expansion of the exoccipital bones contributes to the formation of the lateral buttress of the air-bladder. The transverse processes of the IVth vertebra are decurved and flattened at their outer edges. These meet the post-temporal exoccipital articulation. The lateral walls of the air-bladder are thus supported by these bones. The transverse processes of the IVth vertebra and that of the Vth are joined at their bases by the sutural union and ankylosis of their respective neural arches and spinous processes into a lateral cartilaginous expansion under which the anterior half of the air-bladder rests. The VIth vertebra is the first to bear ribs. There is no 'elastic spring' apparatus common to some Pangasiid genera. The crescentic processes of the tripus is conspicuous and well developed unlike the tripus itself. There are 43 vertebrae, 21 precaudal and 22 post-caudal.

The air-bladder is not very large (Text-fig. 3c) and measures 23 mm. in a specimen of 126 mm. standard length. Internally, the median longitudinal septum divides the bladder incompletely into two lateral compartments. The median transverse septum arises from the upper wall of the bladder and is broadly rooted to the floor. The anterior chamber thus formed communicates freely with the lateral compartments. There are no other divisions.



TEXT-FIG. 1. Anterior vertebral modifications for the support of the air-bladder. $\times 3$. BO = Basioccipital; EXO = Exoccipital; PTM = Post-temporal; IV, V, VI = vertebra.

Bridge and Haddon (1893, pp. 214–221) gave an account of the anterior vertebral modifications of *Pangasius* species for support of the air-bladder. Hora (1937, pp. 235–240) gave these details for *Helicophagus*. Comparison of *Cranoglanis* with *Pangasius* and *Helicophagus* shows that the former differs from the latter two genera in the following features:

1. The inferior limb of the post-temporal bones is simple and small.
2. The transverse processes of the IVth vertebra is not cleft to its root to form distinct anterior and posterior divisions.
3. The air-bladder is simple with no multiple internal divisions.

Hora (*op. cit.*, p. 240) concluded that '*Helicophagus* with long barbels, well developed dentition, without an "elastic spring" apparatus, etc. etc. is at a somewhat higher stage of evolution than *Pangasius*'. *Cranoglanis* although similar to *Helicophagus* in these features, yet differs from both these genera and has the basic Bagrid pattern in its principal osteological features. However, in view of the osteological and other morphological peculiarities discussed earlier, it cannot be included under Bagridae.

Thus it would seem that Myers' provision of a separate family for *Cranoglanis* is fully justified. The family seems to have evolved from Bagrid ancestors such as *Horabagrus* Jayaram (1955) and is at present intermediate between Pangasiidae and Schilbeidae on the one hand and Bagridae on the other.

SYNONYMY AND DESCRIPTION

Family CRANOGLANIDIDAE Myers, 1931

Genus *Cranoglanis* Peters, 1880

- Cranoglanis* Peters, *Mber. Akad. Wiss. Berlin*, 1880, 1030 (original diagnosis; generic type by monotypy *C. sinensis* Peters from 'Hongkong').
- Nichols, *Bull. Amer. Mus. nat. Hist.*, LVIII, 1928, 6 (name only; list of species in AMNH).
- Myers, *Lingnan Sci. J.*, X, Nos. 2 and 3, 1931, 261 (new provisional family Cranoglanididae proposed; affinities discussed).
- Nichols, *Freshw. Fish. China in Nat. Hist. Cent. Asia*, IX, 1943, 38 (data from Peters).
- Pseudeutropichthys* .. Koller, *Ann. naturh. (Mus.) Hofmus. Wien.*, XLI, 1927, 28 (original diagnosis; generic type by monotypy *P. multiradiatus* from Kang Kong, Hainan).

DIAGNOSIS.—

Eyes low set, visible when viewed from below. Snout depressed, head large with a casque of bony, rugose, uncovered shield. Pelvic fin with 12–14 rays. Anal fin with 36–41 rays. Air-bladder large and free.

For detailed characteristics of the genus, reference may be made to the description of the species.

Cranoglanis boudierius (Richardson)

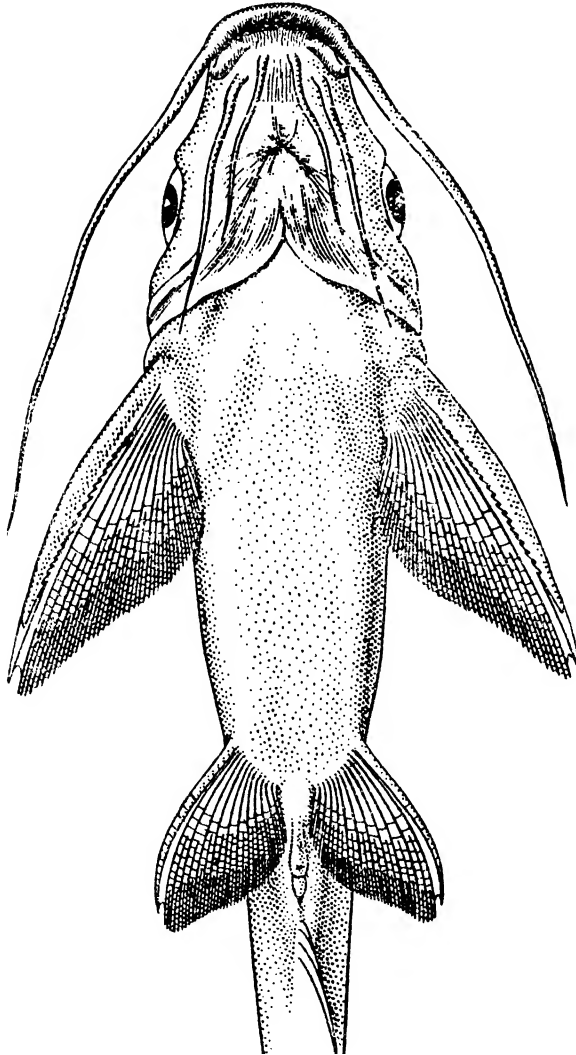
- Bagrus boudierius* .. Richardson, *Rept. Brit. Assn.*, 1845, 283 (description based on a specimen and drawings of Reeves (No. 203) and Hardwicke (No. 183); holotype lost).
- Cranoglanis sinensis* .. Peters, *Mber. Akad. Wiss. Berlin*, 1880, 1030, Fig. 1 (type locality Hongkong; doubted to be an error by Herre, 1934, and revised as Wuchow, Kwangsi province, China).
- Nichols, *Bull. Amer. Mus. nat. Hist.*, LVIII, 1928, 6 (list of specimens in AMNH; not present).
- Rendahl, *Ark. Zool.*, XXA, 1928, No. 1, 171 (systematic position discussed; considered under Ameiuridae).
- Myers, *Lingnan Sci. J.*, X, Nos. 2 and 3, 1931, 262 (name only).
- Herre, *Ibid.*, XIII, 1934, 327 (type locality revised to Wuchow, Kwangsi province, on the basis of specimens examined).
- Nichols, *Freshw. Fish. China in Nat. Hist. Cent. Asia*, IX, 1943, 38 (name only; data from Peters; Herre's revision of type locality quoted).
- Pseudeutropichthys multiradiatus* .. Koller, *Ann. naturh. (Mus.) Hofmus. Wien.*, XLI, 1927, 28, pl. i, Fig. 2 (type locality Kang Kong river, Hainan; description based on three specimens 180, 220 and 300 mm. long).
- Koller, *Anz. Akad. Wiss. Wien.*, LXIII, 1927, 74 (description repeated).
- Myers, *Lingnan Sci. J.*, X, Nos. 2 and 3, 1931, 261 (doubted to be synonymous with *C. sinensis*).

SPECIMENS STUDIED three in total as below:—

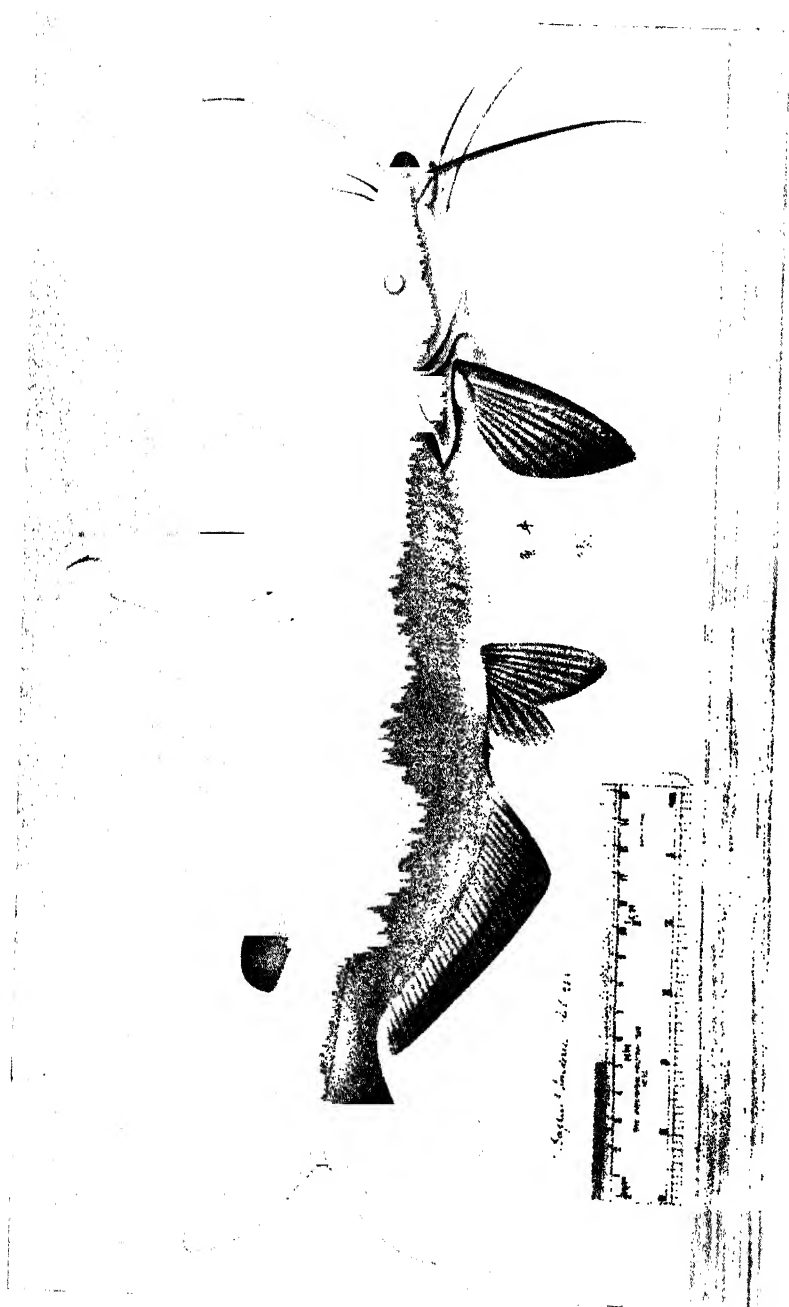
- (1), USNM 94590, 237 mm., Wuchow, Kwangsi province, China, Feb. 12-15, 1934, Herre and Lin coll.
- (2), ZSI F. 11530/1, 118 and 213 mm., Wuchow, Kwangsi province, China, A. W. C. T. Herre coll.

B. VIII; D. I, i, 6; P. I, ii-iii, 9-10; V. iii, 10-11; A. ii, 34-35; C. 23.

Dorsal profile of head forming an angle of about 35 degrees to main axis of body. Supraoccipital and frontal bones exposed and rugose. Length of head 3.49-3.67, depth of body 4.65-4.84, width of head 7.9-8.19, height of head at occiput



TEXT-FIG. 2. Ventral view of head and body. Note the low position of eyes and high count of pelvic fin rays. $\times 4/5$.



Photostat copy of Reeves' illustration (No. 293) of *Panorpa hutchinsoni* Richardson
(courtesy: British Museum of Natural History, London).

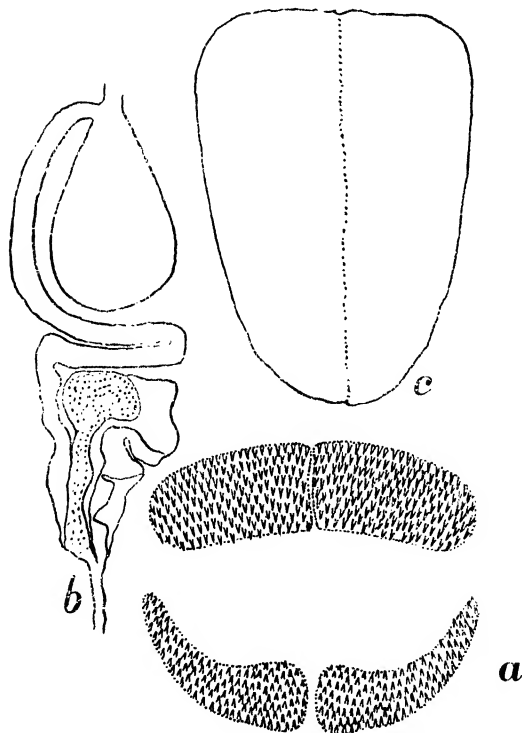
7.65–9.68 times in standard length and 3.91–4.34, 5.78–6.05, 9.83–10.23 and 9.52–12.09 times in total length respectively. Median fontanelle on head does not extend to base of occipital process, which in turn extends as far as basal bone of rayed dorsal fin and is long, broad, exposed and twice as long as broad at base.

Snout obtusely rounded. Lips simple. Mouth moderately wide and oblique. Premaxillary band of teeth villiform, broad, interrupted in middle, and about 3.5 times as long as broad. Mandibular band of teeth also villiform, deeply curved and mesially interrupted (Text-fig. 3a). No teeth on palate. Maxillary barbels extend to base of pectoral fin; other three pairs shorter than head.

Eyes large, low set, nearly in the middle part of head and with orbital rims totally free from eyelids; 4.83–4.86 diameters in length of head, 1.00 in interorbital width and 2.25–2.36 in length of snout.

Gill membranes free from each other and isthmus, deeply notched below chin; gill formula $18 + 13 + 76 = 107$ (as on right anteriormost arch of a specimen 237 mm. long).

Rayed dorsal fin with one spine and seven rays, last ray simple; longest ray extends to adipose fin when depressed. Dorsal spine long, serrated on its inner edge with 10 downward facing teeth and occasionally outer edge also being serrated; dorsal spine 1.00–1.19 times in length of head. Adipose dorsal fin very small, smooth, posteriorly free, separated from rayed dorsal fin by a space about two times as long as base of latter. Pectoral fin with one spine and 11–13 rays, two to three innermost rays simple. Pectoral spine serrated on its inner edge with 10–12 feeble teeth and 1.27–1.33 times in length of head; clavicular processes short, rugose and $\frac{1}{4}$ length of pectoral spine. Pelvic fins reach anal fin base. No pre-anal papilla. Anal fin



TEXT-FIG. 3. a. Dentition. $\times 3$; b. Alimentary canal. $\times 1\frac{1}{2}$; c. Air-bladder. $\times 1\frac{1}{2}$.

very long with 36–41 rays, anteriormost two rays simple and the longest ray does not extend to root of caudal fin. Caudal peduncle 1.40–1.41 times as long as deep. Caudal fin forked but not to its base. Lateral line curved above pectoral fins.

Other proportions and counts presented in Table 1.

TABLE 1
PROPORTIONS AND COUNTS OF *Cranogobius boudierius* (Richardson)

	Range	Mean
Total length	266–295 mm.	280.5
Standard length	213–237 mm.	225.0
Stand. length/Pre-dorsal length	2.37–2.51	2.44
Stand. length/Post-dorsal length	1.58–1.66	1.62
Stand. length/Pre-pelvic distance	1.87–1.97	1.92
Stand. length/Length of max. barbel	2.55–2.62	2.59
Stand. length/Length of dors. spine	3.67–4.16	3.92
Stand. length/Length of caudal ray	4.203–4.44	4.32
Stand. length/Width of anal base	3.07–3.18	3.13
Length of hd./Length of snout	2.06–2.25	2.11
Length of hd./Interorbital width	4.83–4.86	4.85
Length of hd./Width of gape of mouth	3.78–3.87	3.83
Length of hd./Length of max. barbel	Longer than head	
Length of hd./Length of nasal barbel	1.97–2.64	2.31
Length of hd./Length of inner mand. barb.	2.27–2.97	2.62
Length of hd./Length of outer mand. barb.	1.39–1.57	1.48
Length of hd./Length of dorsal fin	1.06–longer	—
Length of hd./Width of dorsal fin base	2.61–2.96	2.79
Length of hd./Length of pectoral fin	1.31–2.29	1.80
Length of hd./Length of pelvic fin	1.93–2.06	1.995
Length of hd./Length of anal fin	2.07–2.19	2.13
Length of hd./Length of caud. peduncle	2.07–2.09	2.08
Length of hd./Height of caud. peduncle	2.70–2.96	2.83
FIN RAYS: Dorsal	7–7	7
Pectoral	11–13	12
Anal	36–41	38.5
Caudal	23–23	23

COLOURATION.—

In spirit preserved specimens, uniformly deep brown on sides and above and pale brown beneath. ZSI specimens are of a lighter hue with a somewhat yellowish tinge.

ALIMENTARY CANAL (Text-fig. 3b).—

The stomach is pear-shaped, muscular, thick walled and with the intestines not much coiled. The three specimens examined had empty stomachs.

DISTRIBUTION.—

Wuchow, Kwangsi province, China; Hongkong and Hainan islands.

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of the British Museum (Natural History) for the loan of specimens and for the information regarding the holotype. Thanks are also due to the NISI for the award of a research fellowship.

ABSTRACT

Myers (1931) established the family Cranoglanididae for a genus of peculiar Chinese catfishes with Pangasiid and Bagrid affinities. In the course of a revision of the fishes of the family Bagridae, the writer found that *Bagrus boudierius* Richardson, previously confused with *Pelteobagrus* species, is referable to *Cranoglanis*. As such, the specific name of the type species *C. sinensis* is changed to *C. boudierius*. Herre's (1934) statement that *Cranoglanis* may not be found in an island is doubted, since Koller's (1927) species *Pseudeutropichthys multiradiatus*, synonymous with *C. boudierius*, is also known from Hainan Island. Osteological features show that the genus *Cranoglanis* has basic Bagrid pattern, but sufficiently different in external characters to warrant a family status as proposed by Myers. A description of the species is given on the basis of available material.

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